Predicting the intramuscular fat content in porcine *M. longissimus* via ultrasound spectral analysis with consideration of structural and compositional traits

Dissertation
zur Erlangung des Doktorgrades
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Referent:	Prof. Dr. Michael Wicke
Korreferent:	Prof. Dr. Kay Raum
Tag der mündlichen Prüfung:	Februar 2011

Shortcuts:

AC	Attenuation compensation
AIB	Apparent integrated backscatter
Alpha (α)	Attenuation
A-Mode	Amplitude-Mode
ANOVA	Analysis of variance
ATPase	Adenosine-triphosphatase
B-Mode	Brightness-Mode
BW	Backfat width
C_{fp}	Cepstral first peak intensity
$C_{fp}(\tau_{fp})$	Cepstral first peak time delay
CT	Connective tissue
DFG	Deutsche Forschungsgemeinschaft
DL	Drip loss
DM	Dry matter
DSC	Differential scanning calorimetry
EC	Electrical conductivity
E.g.	*Exempli gratia*: For example
Eq	Equation
FFT	Fast-fourier transformation
Fig	Figure
FOM	Fat-o-Meat'er
FTG	Fast-twitch glycolytic (muscle fibres)
FTO	Fast-twitch oxidative (muscle fibres)
GC	Gas chromatography
IC	Integrated cepstrum
I.e.	*Id est*: That is
IMCT	Intramuscular connective tissue
IMF	Intramuscular fat
LMP	Lean meat percentage
M	Midband Fit
m	Spectral slope
M. longissimus	Musculus longissimus
MLR	Multiple linear regression
MRI	Magnetic resonance imaging
MUFA	Mono-unsaturated fatty acids
MW	Muscle width
n	Number
NIR	Near-infrared spectroscopy
PBS	Phosphate-buffered saline
PCA	Principal component analysis
PLS	Partial least squares regression
p.m.	Post mortem
PUFA	Poly-unsaturated fatty acids
r	Correlation coefficient
R^2	Coefficient of determination
RF	Radio-frequency
RMSEP	Root mean square errors of prediction
ROC	Radius of curvature

ROI	Region of interest
SAM	Scanning acoustic microscope
SF	Sound field
SFA	Saturated fatty acids
SFC	Sound field correction
SNR	Signal-to-noise ratio
STO	Slow-twitch oxidative (muscle fibres)
Tab	Table
TGC	Time-gain compensation
TOF	Time of flight
UF300	UltraFom 300
US	Ultrasound
v	Velocity
WFCC	Wave front curvature compensation

List of papers:

The following papers are included in this thesis:

Paper I: Koch, T.; Lakshmanan, S.; Brand, S.; Wicke, M.; Raum, K.; Mörlein, D.
„Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle”
Meat Science, accepted: 3. December 2010; DOI: 10.1016/j.meatsci.2010.12.002

Paper II: Koch, T.; Lakshmanan, S.; Brand, S.; Wicke, M.; Raum, K.; Mörlein, D.
„Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: II. Skin and backfat”
Meat Science, accepted: 3. December 2010; DOI: 10.1016/j.meatsci.2010.12.004

Paper III: Lakshmanan, S.; Koch, T.; Brand, S.; Männicke, N.; Wicke, M.; Mörlein, D.; Raum, K. „Prediction of the intramuscular fat content in loin muscle of pig carcasses by quantitative time-resolved ultrasound”
Meat Science, submitted: 15. December 2010

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1 Introduction

Today the certification of quality levels is an essential part of food industry and can be found in nearly every sector. The quality of meat depends on a variety of parameters like nutritional values (proteins, vitamins, etc.), cleanness (micro-organisms, pharmaceuticals, etc.) and sensory characteristics (juiciness, tenderness, etc.). Out of those, the intramuscular fat content (IMF) is widely regarded as one of the major parameters influencing sensory characteristics (Fernandez, Monin, Talmant, Mourot & Lebret, 1999; Wood, Nute, Richardson, Whittington, Southwood, Plastow, Mansbridge, da Costa & Chang, 2004; Suzuki, Irie, Kadowaki, Shibata, Kumagai & Nishida, 2005; Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes & Whittington, 2008). To obtain the best sensory quality, threshold levels from 2 to 3.5 % IMF in porcine *longissimus* muscle have been mentioned (Blanchard, Warkup, Ellis, Willis & Avery, 1999; Fernandez et al., 1999; Kipfmüller, Bodis, Pescheke & Eichinger, 2000). However, recent investigations revealed average IMF levels of about 1 to 1.5 % for German pig populations (Laube, Henning, Brandt, Kallweit & Glodek, 2000; Link, 2007; Warentest, 2008).

To establish a quality based marketing system, the classification of meat according to the IMF level is indispensable. For this purpose, the use of ultrasound (US) was proven to be a fast, cost-effective and non-destructive method. Ultrasound methods are based on the measurement and analysis of tissue specific sound propagation properties, such as velocity and attenuation. Different investigations stated the practicability of B-mode image analysis to predict the IMF on living steers reaching R^2 values up to .75 (Hassen, Wilson, Amin, Rouse & Hays, 2001; Hassen, Wilson & Rouse, 2003). Contrary, measurements performed at living pigs were less predictive not exceeding R^2 values of .48 (Newcom, Baas & Lampe, 2002; Maignel, Daigle, Gariépy, Wilson & Sullivan, 2010).

Compared to those measurements, an improvement could be realized analyzing the spectral data of ultrasound measurements, which contain more comprehensive information about tissue constitution (Park, Whittaker, Miller & Bray, 1994a; Lizzi, Feleppa, Alam & Deng, 2003). In medical research, spectral analysis is already widely used for enhanced tissue characterisation or the detection of malignant alterations (Nair, Kuban, Obuchowski & Vince, 2001; Oelze, Zachary & O'Brien, 2002; Scheipers, Ermert, Sommerfeld, Garcia-Schürmann, Senge & Philippou, 2003). However, investigations describing spectral analysis in porcine muscle are comparably rare. In a previous study spectral analysis of unprocessed backscatter

signals, obtained with a sophisticated diagnostic ultrasound device, was used to non-invasively estimate the intramuscular fat content of porcine loin muscle, reaching an average prediction error of 0.36 % (Mörlein, Rosner, Brand, Jenderka & Wicke, 2005a). However, both the instrument and the prediction errors were not yet satisfying for industrial use at slaughter.

The exact knowledge of the sound properties at the region of interest (ROI) and of tissues passed by the radio frequency (RF) signal is a prerequisite of a reliable ultrasound spectral analysis. These properties are determined by the system (e.g. sound field), but also by the tissues along the sound propagation path (Gärtner, 2001). The incorporation of sophisticated calibration and correction methods are therefore anticipated to improve the accuracy of ultrasound based IMF predictions (Brand, Mörlein & Rosner, 2002). Unfortunately, reference data for backfat or muscle published so far (Chivers and Parry, 1978; Greenleaf, 1986) mostly differ in one or more of the required conditions (e.g. temperature, species, measurement direction) and are therefore not assignable to porcine hot carcass conditions early post mortem.

Several investigations have shown the feasibility of high frequency ultrasound measurements (up to the GHz range) with a scanning acoustic microscopy (SAM) to investigate biological tissue (Daft, Briggs, 1989; Saijo, Sasaki, Okawai, Nitta, Tanaka, 1998; Raum, 2008). Thus, the target of the present investigation is to collect *in-vitro* reference data in a set of pig carcasses typically found in German slaughter facilities. The measurements will be conducted using a scanning acoustic microscope. Environmental conditions (e.g. temperature, measurement angle) will be chosen in accordance to measurements at suspended carcasses. The obtained acoustic parameters will be related to compositional and structural traits to evaluate their potential influence on IMF prediction. The new reference values will be used for calibration and correction algorithms anticipated to improve the accuracy of ultrasound based IMF prediction on hot porcine carcasses. Finally, the improved correction algorithms will be implemented into a modified hand-held ultrasound device and measurements will be conducted at a representative set of pig carcasses early post mortem.

2 Background

The following section is intended to give an overview about investigations and publications targeting on muscle and backfat composition as well as relationships between them and meat quality traits. Furthermore, a brief review is given about the principles and use of ultrasound for tissue characterization and its advantages and disadvantages.

2.1 Muscle structure and composition

On an average the muscle consists of about 75 % water, 19 % protein, 0.5 – 8 % lipids and 1 % glycogen and is composed of several tissues including muscle fibres, connective tissue, intramuscular fat, vascular and nervous tissue (Lefaucheur, 2010). The total amount of protein can be divided into 3 groups. The majority (about 50 – 55 %) is used for myofibrils while the sarcoplasmatic proteins account for about 30 – 34 %. Finally, the remaining 10 – 15 % are connective tissue proteins (Tornberg, 2005). As a result of this large variety, the muscle itself is a highly heterogeneous tissue with numerous factors proven or anticipated to affect meat quality. This includes not only compositional variables like the amount of IMF and the connective tissue but also the size and distribution of muscle fibres (and therefore muscle thickness). In the following, some aspects of muscle structure and their relationships to meat quality are summarized.

2.1.1 Muscle fibres

Muscle fibres represent about 90 % of the muscle volume and are organized in form of bundles divided against each other with connective tissue. Several methods have been introduced to differentiate between fibre types including metabolic properties and differentiation of myosin heavy chains (Peter, Barnard, Edgerton, Gillespie & Stempel, 1972; Hintz, Coyle, Kaiser, Chi & Lowry, 1984). However, one of the most prominent ways today is histochemical characterization using succinate dehydrogenase and reversed ATPase staining (Horák, 1983). Here, 3 types of fibres with differing size, metabolic and contractile properties are divided against each other. These types are fast-twitch oxidative (FTO), slow-twitch oxidative (STO) and fast-twitch glykolytic (FTG) fibres with FTG fibres reaching a total amount of about 75 % of all fibres in porcine muscle (Fiedler, Ender, Wicke, Maak,

Lengerken & Meyer, 1999). STO fibres exhibit low ATPase (adenosine-triphosphatase) activities and possess a low quantity of glycogen, while they are rich in myoglobine and are sustained for low intensity contractions for basic movement. FTG fibres show high ATPase activity, are rich in glycogen and low in myoglobine and are used for short, intense movement and a fast contraction. Furthermore, as shown via liquid chromatography after chloroform / methanol extraction, the amount of phospholipids (the main constituents of the cell membrane) is about 30 % higher in oxidative fibres, contributing to the total IMF with about 0.7 – 0.9 % (Leseigneur-Meynier and Gandemer, 1991).

The total amount of muscle fibres is constant after birth but the diameter of the fibres (accompanied with the total muscle thickness) increases during maturation (Zgur, 1991). The distribution of the fibre types is mostly consistent but can be influenced by training and/or rearing conditions, inducing a transformation between glykolytic and aerobic fibre types to a certain extent (Wicke, 1989; Gondret, Combes, Lefaucheur & Lebret, 2005). Additionally a wide range of factors (e.g. sex, breed, or halothane genotype) have been stated to influence the muscle fibre composition (Karlsson, Klont & Fernandez, 1999).

2.1.2 Connective tissue

A large variety of investigations targeted on the structure and composition of the intramuscular connective tissue (IMCT) (Mayne and Sanderson, 1985; Purslow, 2002; Purslow, 2005). The IMCT mainly consists of collagen- and elastin-fibres surrounded by a matrix of proteoglycan and is present in muscles ranging from 1.5 to about 10 % of dry matter weight (Lepetit, 2008). The proteoglycan matrix itself has been shown to influence ($r = .49$) the shear force of raw meat in porcine muscle (Nishiumi, Kunishima, Nishimura & Yoshida, 1995).

For the IMCT, mainly three different hierarchical domains can be stated differentiated after their function and position: the endomysium is a thin layer enveloping individual muscle fibres. It can easily reorientate with changing muscle length, and the average diameter of the collagen fibrils is about 48 nm. The perimysium, providing the majority of total connective tissue, separates each muscle into muscle bundles surrounding a varying number of muscle fibres. The perimysial layers can be divided into primary (large fascicles) and secondary (small fascicles) ones with a fibril diameter of about 65 – 67 nm and are mostly aligned in a honeycomb structure (Nishimura, Hattori & Takahashi, 1999; Fang, Nishimura & Takahashi, 1999). As for the endomysium the fibres can easily reorientate, which is important for muscle

contraction. Finally, the epimysium separates individual muscles and the collagen fibres are close-packed (Purslow, 2005). Beneath structural differences there are also age and breed related differences. The endomysium becomes thicker and denser during growth, while the amount of covalent crosslinks increases, linking individual collagen molecules together. This is expected to be one of the major factors causing a decreased tenderness of porcine muscle with increasing age (Fang et al., 1999; Lefaucheur, 2010).

2.1.3 Intramuscular fat

As the IMF is considered as one of the main quality parameters, it has widely been investigated during the last years. Currently, values as low as 1 to 1.5 % IMF have been stated for German pig populations with few animals reaching 2 % (Laube et al., 2000; Link, 2007; Warentest, 2008). This is mostly in accordance with international results where comparable IMF values were found, except for Duroc crossbreeds reaching higher IMF values (Blanchard et al., 1999; van Laack, Stevens & Stalder, 2001). The majority of the IMF is located in intramuscular adipocytes grouped along or within the perimysium, while a small amount exists in form of phospholipids inside the cell membrane of muscle fibres (Essen-Gustavsson, Karlsson, Lundström & Enfält, 1994).

The IMF has been found to be highly influenced by several factors like breed, feeding regime, gender and age. Higher amounts of IMF and better sensory results for pigs have been stated for animals fed with high energy (Blanchard et al., 1999) or fat enriched food (Lengerken, Schröder, Haugwitz, Berger & Siegel, 1984). An increasing amount of IMF with increasing age was published (Wagner, Schinckel, Chen, Forrest & Coe, 1999), which is partially based on the fact that IMF is deposited last compared to the increasing thickness of the backfat layers (Kolstad, 2001). Differences in gender have been mentioned with boars having the lowest amounts of IMF (Lengerken, Bergmann & Pfeiffer, 1989) followed by sows, while castrates reach the highest values (Unruh, Friesen, Stuewe, Dunn, Nelssen, Goodband & Tokach, 1996). Furthermore, significant differences are known both longitudinal and cross-sectional the *longissimus* muscle. Lowest quantities of IMF can be found at the 2nd / 3rd last rib showing increasing values for both the cranial and caudal end (Heylen, 1999). In addition, Heylen (1999) stated significant variations of more than 0.7 % IMF comparing 5 cross-sectional regions of porcine chops with highest values in the ventral region.

Comparing given IMF values, attention has to be paid as the obtained results may vary due to differences in the used methods. Except new methods which are still under investigation (e.g. X-ray-computed tomography or magnetic resonance imaging), mainly two methods have been implemented during the last years. Measurement using Soxhlet extraction is widely regarded as reference method, determining the IMF directly via lipid extraction. However, methodical differences like the polarity of the solvent and the use of acid pre-treatment may change the obtained results significantly. The use of petrol ether extraction without acid treatment for example, results in values approximately 0.3 to 0.6 % IMF lower compared to the same analysis with acid treatment (Reichard, Müller, Schuster & Peschke, 1998).

IMF results comparable to chemical determination can be obtained using measurements at homogenized samples. Examples are near-infrared spectroscopy (NIR) reaching high correlations (r = .90) to Soxhlet extraction (Alomar, Gallo, Castaneda & Fuchslocher, 2003; Hildrum, Wold, Vegard, Renou & Dufour, 2006) or magnetic resonance imaging (MRI) at rinsed bovine meat (Sorland, Larsen, Lundby, Rudi & Guiheneuf, 2004) reaching R^2 values up to .98 compared to Foss-let extraction. However, as for the chemical analysis several factors like the sample preparation, temperature and degree of homogenization may influence the results.

All the above mentioned methods however have one or more disadvantages reducing their use at slaughter. Some require a large stationary build up (e.g. computed tomography) and are comparably cost- and time-expensive and therefore mainly of scientific use. Others perform measurement at homogenized muscle samples (NIR, MRI) and therefore reduce the carcass value. By now, none of the methods provide a cheap, fast and non-destructive IMF determination directly on the carcass immediately after slaughter.

2.1.4 Influence of muscle structure and composition on meat quality

As mentioned before, IMF is widely known to affect sensory characteristics and quality of meat. Several investigations stated increased taste and juiciness with increasing IMF. However, these relationships do not seem to be linear. Most publications suggest a threshold level of about 2 to 3.5 % (determined via Soxhlet extraction with acid pre-treatment) in the *longissimus* muscle at the 2^{nd} / 3^{rd} last rib as optimal for the taste of porcine meat (Blanchard et al., 1999; Fernandez et al., 1999; Kipfmüller et al., 2000). Slight

differences in the results can be reasoned in a genetic influence of IMF level and marbling (IMF fleck size and distribution) required for optimal taste (Josell, von Seth & Tornberg, 2003; Faucitano, Huff, Teuscher, Gariepy & Wegner, 2005). Nonetheless, most studies agree that IMF values higher than 3.5 % do not result in a further improvement of sensory characteristics or even alter them (Fernandez et al., 1999).

Besides the IMF, IMCT is regarded as one of the major parameters responsible for meat tenderness (Purslow, 2005). Investigations performed on a wide range of carcasses stated correlations up to r = .98 between the collagen content and shear-force values (Liu, Nishimura & Takahashi, 1996; Fang et al., 1999; Dransfield, Martin, Bauchart, Abouelkaram, Lepetit & Culioli, 2003). An increase in thickness, especially for the perimysium and the endomysium, has been stated for the porcine *semitendinosus* muscle from 0 to 56 months of age with highest increases in the first six months (Fang et al., 1999). The amount of IMCT is suggested to be mainly responsible for the age related decrease of tenderness in pigs and an increased thickness has been stated to increase shear force values (Purslow, 2005). Out of the different layers the perimysium has been shown to be the strongest and most dominant in terms of meat tenderness (Lewis and Purslow, 1990).

Furthermore, the relative proportion, amount and medium diameter of muscle fibres have been found to influence many aspects of meat quality including water holding capacity, tenderness and juiciness (Karlsson et al., 1999; Ryu and Kim, 2005; Lefaucheur, 2010). Poor meat quality has been mentioned to occur with both extreme numbers and size of myofibres (Rehfeldt, Tuchscherer, Hartung & Kuhn, 2007). Other investigations stated that a higher total fibre amount combined with lower amounts of FTG fibres result in higher meat quality (Kim, Lee, Choi, Kim, Yoo & Hong, 2008). The increasing proportion of FTG fibres has been mentioned to increase the post mortem pH decline and protein denaturation while the water holding capacity decreases (Ryu and Kim, 2005; Choe, Choi, Lee, Shin, Ryu & Hong, 2008). In addition, a higher amount of FTO fibres decreases water holding capacity, tenderness and flavour (Henckel, Oksbjerg, Erlandsen, Barton-Gade & Bejerholm, 1997). However, compared to statements targeting IMF or IMCT, relations between histological traits and meat quality are less-well understood and their influence on meat quality may partially be reasoned in correlations with other compositional parameters (e.g. IMCT, IMF).

2.2 Backfat and skin

Ultrasound measurements performed on hanging carcasses differ significantly from measurements performed on hashed meat or even the shear muscle. One of the alterations arises when the signal passes the backfat layer on its way to the muscle as described in 2.3.4. Many of the used ultrasound systems consider the complete backfat as one homogeneous tissue or separate only for skin and fat. The following section will show that this assumption does not coincide with the real backfat composition. A more accurate analysis of individual layers and corresponding ultrasound parameters may help to increase the understanding of how the US signal is influenced by passing the backfat prior to muscle analysis.

2.2.1 Structure and composition

The backfat as defined in the present study is build up out of the skin and 3 individual fat layers. All fat layers consist out of water, collagen and lipids, while the total amount of all 3 constituent parts differs depending on age, feeding regime, breed or backfat thickness. Overall, water accounts for about 10 to 25 %, collagen for 2 to 5 % and the lipids adjust the majority with about 65 to 85 % (Moody and Zobrisky, 1966; Wood, Enser, Whittington, Moncrieff & Kempster, 1989). However, the composition also differs between the 3 individual fat layers. While the outer (subcutaneous) and the middle (intermediate) layers are comparable to each other, the inner layer has significantly lower amounts of fatty acids and higher amounts of water (Moody and Zobrisky, 1966; Fortin, 1986). Those differences can partially be explained by the development of the fat layers. While the subcutaneous fat layer is more dominant and thickest at light weights, the intermediate layer develops fastest of all 3 layers and is thus more dominant in heavy animals. The inner layer is developed last and shows the lowest overall thickness (Moody and Zobrisky, 1966; Fortin, 1986). The amount of fatty acids increases with the thickness of the fat layer as more fatty acids are deposited during growth, resulting in the lowest overall amount of fatty acids in the inner layer (Moody and Zobrisky, 1966).

The skin of all mammalians consists mainly of collagen accounting for about 75 % of dry matter weight. Both structure and composition (e.g. compactness) of collagen however, have been shown to differ not only between pig breeds but also between different body regions (Meyer, Neurand & Radke, 1982). As the mechanical properties of the dermis highly

depend on the rigid and stable collagen network (Mowafy and Cassens, 1975), they can also be expected to differ between breeds. Comparable findings have been mentioned for the amount and positioning of hair follicles (Mowafy and Cassens, 1975).

2.2.2 Fatty acids

All fatty acids can be divided into 3 major groups by the number of their double bonds (i.e. their level of unsaturation). Earlier investigations at German pig populations stated that the majority of the fatty acids are saturated fatty acids (SFA ~ 37 %) or mono-unsaturated fatty acids (MUFA ~ 44 %). The remaining 19 % belong to the poly-unsaturated fatty acids (PUFA) (Link, 2007). As for the overall composition, fatty acid distribution is also largely affected by breed type, production system or (to a great extend) diet (Ninoles, Clemente, Ventanas & Benedito, 2007; Daza, Ruiz-Carrascal, Olivares, Menoyo & López-Bote, 2007). However, even if quantity and distribution of the fatty acids differ, few of them occur in amounts of more than 10 %. Oleic acid accounts for about 40 %, followed by palmitic acid (~ 25 %), linoleic and stearic acid (each about 10 % to 15 %) (Wood et al., 1989; Davanel, Riaublanc, Marchal & Gandemer, 1999).

As for the total amount of fatty acids, the distribution also varies with the thickness of the layers. The amount of SFA increases with increasing fat depth while the amount of PUFA decreases (Wood, 1973; Villegas, Hedrick, Veum, McFate & Bailey, 1973; Lo Fiego, Santoro, Macchioni & De Leonibus, 2005).

2.3 Ultrasound

First experiments using ultrasound for animal breeding purposes (e.g. carcass composition) have been conducted decades ago (Müller-Haye, 1965). Today, ultrasound is one of the most prominent methods able to investigate biological tissue in a fast and non-destructive way and is widely used in medical research (Oelze et al., 2002; Lizzi et al., 2003; Gelse, Olk, Eichhorn, Swoboda, Schoene & Raum, 2010). Concurrent to medical developments, its use to determine muscle or backfat thickness on carcasses immediately after slaughter became more and more prominent (Broendum, Egebo, Agerskov & Busk, 1998). In addition, wide varieties of methods have been introduced (and are still under investigation)

trying to determine the structural composition (e.g. IMF) of the muscle. The following chapter will give an overview about the opportunities and drawbacks of ultrasound in terms of muscle composition prediction.

2.3.1 Principals

Ultrasound is defined as an acoustic wave above the human acoustic range of 20 kHz, even if only the range between 2 and 100 MHz is used for medical diagnostics. The ultrasound signal itself is generated using the reciprocal piezoelectric effect: Some polar materials (e.g. quartz, man-made piezo-ceramics) generate an electrical potential if applied to mechanical stress (e.g. pressure). This is called the piezoelectric effect. On the contrary, the generation of an electrical field results in mechanical deformations. If alternating voltage is used as electrical field, a periodic deformation is generated and this wave is devolved to the adjacent tissue. The wave propagates through the tissue and gets partly scattered or even reflected at areas with different acoustic characteristics. In turn, the reflection is detected by the transducer and transformed into an electrical signal using the piezo-electrical effect. This obtained signal is then analysed and used to build an image. However, this is true only for pulse-reflection measurements where both, transmitter and detector are a single element. As an alternative method individual components can be used as transmitter and detector and the transmitted signal gets detected instead of the reflected one. This is called the pulse-transmission measurement.

Different methods are used to proceed with the obtained signal. For the amplitude-mode (A-mode) the signal is displayed with the time on the X-axis and the amplitude (the strength of the reflection) on the Y-axis. Using the brightness-mode (B-mode) multiple adjacent A-lines are analysed obtaining a grey image with the time (or depth of the tissue) on the Y-axis and the regional information on the X-axis (Fig. 1). Different acoustic properties caused by different tissues are displayed by differences in the grey-value.

The used ultrasound signal itself can be described by the three parameters frequency, wavelength and amplitude: The frequency is the number of oscillations the wave performs in one second given in hertz (Hz). As mentioned above, ultrasound is defined to use at least 20 kHz (20000 Hz). The wavelength (λ) is defined as the distance between two consecutive oscillations (given in µm). Finally, the amplitude is the strength of the sound signal. It is given as a relative value, e.g. how much of a pulse decreases as it passes through the tissue.

This is specified in units of decibels (dB) where a difference of the factor 2 is related to 6 dB. As described in the next chapter, the maximum depth the signal can travel depends on the used frequency. However, an increase in frequency also increases the lateral resolution and therefore the ability to differentiate between multiple structures in lateral direction. Therefore, the frequency always has to be adapted to the tissue and the required signal range.

2.3.2 Sound velocity and attenuation

The most commonly obtained parameters using US measurements are sound velocity and attenuation. Sound velocity (given as ms^{-1}) describes the speed of the sound wave inside the tissue. While it is easy to measure the sound velocity in samples of known thickness (Benedito, Carcel, Rossello & Mulet, 2001) velocity values can only be assumed for measurements of unknown thickness (e.g. hanging carcasses). An important role approaches to the sound velocity as it is required for accurate sound field correction (see 2.3.4) and thus an accurate knowledge of velocity values of the passed tissue is important.

The second important parameter, the attenuation (given as dB MHz^{-1} cm^{-1}), is the weakening of the ultrasound beam (dB) in relation to the thickness of the passed medium (cm^{-1}) and the used frequency (MHz^{-1}). As for sound velocity, knowledge of attenuation is important as the sound field (the area inside the medium where the sound waves propagate; Fig. 1) and signal strength varies in tissues with different attenuation values. If the attenuation of a medium is known (or calculated) the input signal amplitude can be compensated for any loss of energy at the desired imaging depth. Attenuation can be calculated from the gated radio-frequency signal within the region of interest (ROI) after normalizing the spectrum with the corresponding modified reference spectrum.

2.3.3 Spectral analysis

Contrary to grey-scale image analysis or time of flight measurements, ultrasound spectral analysis is suggested to provide more comprehensive information about tissue constitution (Lizzi et al., 2003). However, by now only few investigations were performed targeting meat quality parameters, while even less were realized on porcine meat. Early investigations using spectral analysis for meat quality purposes were performed directly at beef muscle without backfat or skin resulting in R^2 values of 0.33 (Amin, Patel, Roberts,

Rouse & Wilson, 1995). Further investigations confirmed relationships between attenuation (r = .60) or backscatter intensity (r = .71) with fat content under laboratory conditions (Abou El Karam, Suchorski, Buquet, Berge, Culioli, Delachartre & Basset, 2000). The best results could be obtained at beef via A-mode measurement explaining about 82 % of the IMF variation at small, excised muscle samples (Park et al., 1994a). This is contrary to early investigations performed at porcine muscle where no relationships between the IMF and any of the spectral parameters could be found (Ville, Rombouts, Van Hecke, Perremans, Maes, Spincemaille & Geers, 1997). However, this may be due to nonconforming calibration of the US device or the small amount of muscle (1g) used for chemical IMF determination.

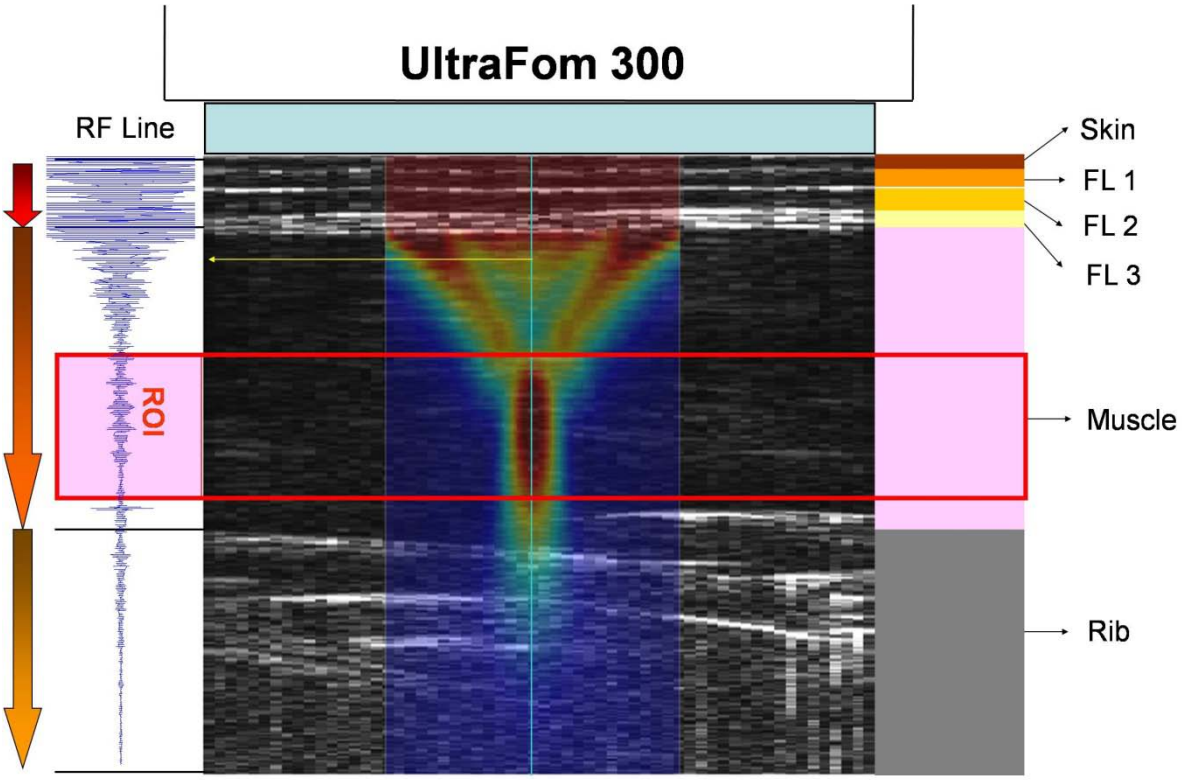


Fig. 1: B-mode image of the *musculus longissimus* and the colour coded acoustic focus of the UltraFom300 ultrasound system. Radio frequency (RF) signals are attenuated and reflected while passing through the medium. [ROI: Region of Interest, FL: Fat layer]

To obtain a spectrum, the RF-signal (Fig. 1) is first multiplied with a specific gate-function (e.g. Hanning) to improve the signal-to-noise ratio (the ratio of the signal power compared to the noise corrupting the signal) (Fig.2). Thereafter, a fast-fourier transformation (FFT) converts the obtained time-signal into the frequency spectrum (Fig.3). In addition, correction functions (e.g. comparison with reference spectrum) have to be applied and will be discussed in more detail in the following chapter.

The general shape of the obtained spectrum (e.g. frequency dependence, amplitude) is influenced by structures (scatterers) smaller than the wavelength and is determine by size, shape, distribution and impedance (defined as the product of density and sound velocity) of the scatterers. Observed parameters include spectral slope (dB MHz^{-1}), apparent integrated backscatter (dB) and midband fit (dB), describing form (slope) and the regression line at the centre frequency of the obtained power spectrum (Fig.3) (Park et al., 1994a; Amin et al., 1995; Lizzi, Alam, Mikaelian, Lee & Feleppa, 2006). Out of those parameters the spectral slope is expected to be influenced by scatterer size, while the integrated backscatter is influenced by differences of the acoustic impedance and distribution of the scatterer. Furthermore, all spectral parameters are affected by attenuation while Midband fit and integrated backscatter values are related to each other (Lizzi, Astor, Liu, Deng, Coleman & Silverman, 1997).

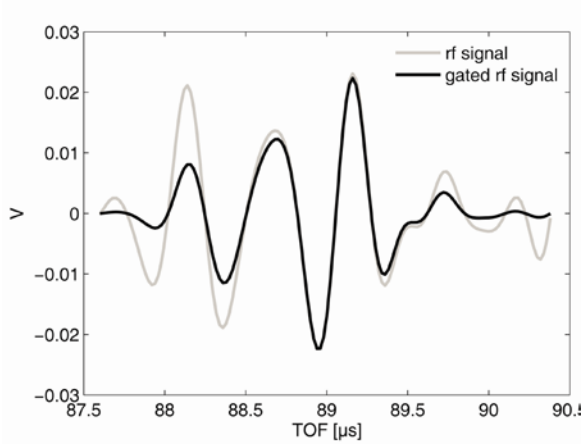


Fig.2: Gated RF signal from the region of interest; TOF = time of flight

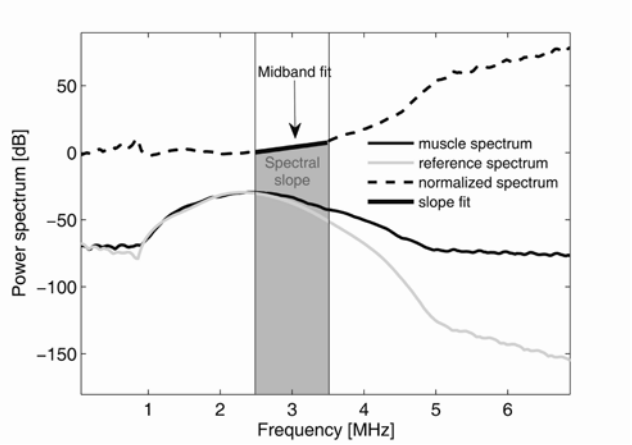


Fig.3: Spectral parameters estimated from normalized power spectrum

Even if the use of spectral analysis increases the amount of information, the method still has some error susceptibilities. The FFT calculated spectra consist of a multiplication of the US impulse and the distribution of the scatterer. The results can easily be analyzed if few homogenous scatterers are investigated. However, scatterers of biological tissues often appear in a random distribution and the US signal is therefore scattered by a multitude of them. As a result, the detected signals consist of a mixture of several functions which can be hard to identify. Furthermore, the obtained parameters are affected by system specific effects (time gain compensation (TGC), sound field geometry (Fig. 1)) and sound propagation effects (sound velocity and attenuation alterations in backfat and intermediate muscle) which have to be corrected for (Gärtner, 2001).

As a further feature of the spectral parameters, the cepstrum can be calculated which is defined as the logarithm of the inverse fourier-transformed spectrum. It is calculated by computing the logarithm of the spectrum, which converts the multiplication of the US signals into additions. A thereafter performed FFT enables to divide the signals obtained by different scatteres displayed in the cepstrum, if the distance between them is larger than the wavelength.

In an earlier investigation spectral and cepstral analysis of unprocessed backscatter signals obtained with a sophisticated diagnostic ultrasound device were used to estimate IMF content of porcine loin muscle (Mörlein, Rosner, Brand, Jenderka & Wicke, 2005b). The measurements were performed on 115 carcasses 45 min post mortem. Acoustic parameters like attenuation, backscatter and cepstral structure were calculated and related to the IMF content. Multivariate prediction of IMF ($R^2 \sim .6$) yielded average prediction errors of 0.36 % and demonstrated the feasibility of ultrasound spectral and cepstral analysis for non-destructive IMF evaluation.

2.3.4 System specific effects and their correction

Studies performed in 10 laboratories revealed highly different backscatter data for measurements on identical phantoms (Madsen, Dong, Frank, Garra, Wear, Wilson, Zagzebski, Miller, Shung, Wang, Feleppa, Liu, O'Brien, Topp, Sanghvi, Zaitsev, Hall, Fowlkes, Kripfgans & Miller, 1999) making comparisons between parameters determined by different systems and users questionable. In addition, significant differences were stated between different users predicting the IMF using the same US-system (Herring, Kriese, Bertrand & Crouch, 1998). To improve the comparability, every sort of ultrasound analysis is therefore intended to make its statement over the investigated tissue independent of the used ultrasound device. To enable that, improved corrections for system specific affects like sound-field and transmission-settings are required.

The general shape of the sound field (e.g. the position of the focus zone with the highest lateral resolution) is given by the used transducer. If measurements are performed in water, the intended sound field is highly comparable with the real one. However, even if the sound velocity of water is comparable to biological tissue, the absence of scatterers makes large differences in terms of US measurements. As a result, travelling of the US signal through biological tissue highly alters the assumed sound field affecting the obtained results

(Gärtner, 2001). This is especially true if the tissue consists of different layers with different values for speed of sound. Thus, the measured sound field in water can only be an approximation of the real sound pressure distribution; the direct measurement of a sound field for a particular transducer inside the carcass is not possible. Therefore, measurements are performed at homogeneous phantoms with known US properties (e.g. sound velocity, attenuation, temperature) which are intended to be comparable to the investigated tissue (Insana, Zagzebski & Madsen, 1983). In the following, spectra are calculated in the same way as for the investigated tissue. The obtained results can then be used as a reference for measurements at biological tissue. However, as even the best phantoms are not able to perfectly simulate the variety of biological tissues the used phantoms are still approximations.

Another correction method is the time gain compensation. Every biological tissue causes a decrease of the signal when passing due to absorption, scattering and reflection. This results in weaker US signals in deeper areas of the investigated tissue. The TGC equals this attenuation and ensures an even brightness for the whole B-mode image by an increasing amplification of the signal with increasing time of the single pulse-echoes. The effect of this TGC has to be corrected prior to the spectral analysis to avoid alteration of the obtained RF signal. Dependent on the used US system the TGC can either be implemented in the system or adjusted by a function generator. In both cases a calibration with either a second transducer or a backscatter phantom is required for the correction (Gärtner, 2001).

All correction methods have in common that they require exact knowledge of the used ultrasound device and the investigated tissue. Misinterpretation of the sound field for example may lead to inferior spatial resolution and signal-to-noise ratios due to misplacement of the region of interest. Therefore, accurate ultrasound and attenuation values of the investigated and trespassed tissues are required to ensure sufficient correction algorithms. Unfortunately, so far no investigation provided datasets for porcine tissue measured under conditions comparable to suspended carcasses early post mortem (Greenleaf, 1986). Investigations on the muscle were mostly performed with a constant fibre direction (Chivers and Parry, 1978) or on bovine muscles (Smith, 1996). Measurements at backfat or skin were conducted at differing temperatures (Ninoles, Clemente, Ventanas & Benedito, 2007) or at as few as 2 animals (Cantrell, Goans & Roswell, 1978). Adequate acoustic parameters for both porcine muscle and backfat under hot carcass conditions have not been provided, so far.

2.3.5 Scanning acoustic microscopy

A specific form of ultrasound measurement is the scanning acoustic microscopy (SAM). Here, the ultrasound frequency is increased up to the gigahertz range resulting in a higher spatial resolution. As a disadvantage of the high frequency, the sample thickness has to be lower compared to a diagnostic US device due to the correspondingly high attenuation. Furthermore, only small areas of the sample can be investigated at once. To obtain high-resolution maps of small tissue samples, the transducer has to be moved slightly after each measurement and the insonification has to be repeated for the whole region of interest. As in all US measurements differentiation between individual tissue components is achieved by reflections of the sound wave due to different elastic conditions. The resolution of the map is limited by the step width of the transducer and the used frequency.

One of the advantages of SAM measurements is the ability to achieve exact values for sound velocity and attenuation inside the sample. The time of flight (TOF) of the US signal inside the sample can easily be compared with reference measurements performed simultaneously in a coupling fluid (e.g. water or phosphate-buffered saline). Acoustic parameters like sound velocity are directly linked with mass density and elastic properties of the tissue and can be calculated from the obtained TOF data. Investigations using high-frequency ultrasound have been proven to be sensitive to changes of the elastic tissue state and several empirical backscatter and attenuation parameters have been proposed to have a predictive potential (Daft and Briggs, 1989; Saijo, Sasaki, Okawai, Nitta & Tanaka, 1998). By now, different groups used ultrasound in the GHz range for medical diagnostics (Jorgensen, Assentoft, Knauss, Gregersen & Briggs, 2001; Raum, 2008). Jorgensen (2001) detected differences of acoustic impedance and speed of sound in different layers of the intestine walls (mucosa, submucosa, circular and longitudinal muscle). Time-related changes in ultrasonic attenuation in rat myocardium during chemical fixation have also been observed (Hall, Dent, Scott & Wickline, 2000). Other studies reported age related changes of collagen content in myocardium and corresponding increase of backscatter and attenuation parameters (Nguyen, Hall, Scott, Zhu, Marsh & Wickline, 2001). However, by now no investigations have been performed completely covering the whole range of tissue characteristics (e.g. muscle and backfat) occurring on hot porcine carcasses (e.g. in terms of temperature or measurement direction).

2.3.6 Ultrasound tissue characterization

A wide range of investigations using ultrasound to predict tissue composition (e.g. fat or water content) have already been published. Early works either used ultrasonic velocity measurement (Whittaker, Park, Thane, Miller & Savell, 1992; Benedito, Carcel, Rossello & Mulet, 2001) or the analysis of digitised B-mode images (Brethour, 1994; Kim, Amin, Wilson, Rouse & Udpa, 1998; Hassen, Wilson, Amin, Rouse & Hays, 2001). The ultrasound propagation speed was reported to correlate with different compositional parameters. Investigations performed on hashed meat with a wide range of compositions (e.g. 3 - 90 % fat) at two different temperatures could explain 99.6 % of the fat variance, 98.7 % of moisture and 85.4 % of protein (Benedito, Carcel, Rossello & Mulet, 2001). However, the investigated IMF, protein and moisture ranges were far from being representative for biological tissue and transferability on carcass measurements is at least questionable. Studies analysing sound velocity values over a specific frequency range found correlations up to -.82 measured at excised beef chops with a constant thickness of 30 mm (Park, Whittaker, Miller & Hale, 1994). Those investigations however, were performed either on solely muscle or hashed meat. Furthermore, the precision of ultrasonic velocity measurements requires a careful setup including thickness determination and temperature adjustment of the investigated tissue (Whittaker, Park, Thane, Miller & Savell, 1992; Park et al., 1994b). Therefore, this method does not appear to be of practical use at slaughter facilities.

Another option determining mainly the IMF content inside the muscle is the analysis of grey-scale B-mode images. In principle, the obtained radio frequency signal is displayed as a grey-scaled speckle image (Fig. 1) due to differences in the acoustic properties between the fat and the surrounding tissue. Specific statistical methods (e.g. Fourier-based and histogram based image analysis) can be applied to quantify the amount of IMF. These methods were successfully applied to predict IMF at living steers with R^2 values up to .75 (Hassen et al., 2001; Hassen et al., 2003) and slightly lower correlations on hot carcasses (Whittaker et al., 1992). Comparable methods performed at living pigs have shown to be less feasible, not exceeding an R^2 value of .48 (Ville et al., 1997; Newcom et al., 2002; Maignel et al., 2010). This is suggested to be due to structural and compositional differences, such as the considerably wider range of IMF in bovine muscle compared to pork loin (Hassen et al., 2001). A large disadvantage of this method is the fact that a majority of the information included in the backscattered signal is wasted as only the amplitude of the signal is used to determine the grey-scale. Furthermore, the speckle texture is affected not only by acoustic

properties of the tissue itself, but also by the used B-mode system, e.g. the time-gain-compensation settings or application of image filters for enhancing the image quality (Jenderka, Gärtner, Zacharias, Heynemann & Cobet, 1999).

Besides the prediction of muscle composition, ultrasound was used to determine the characteristics of oils and backfat layers. An increased sound velocity was stated for backfat batches with higher proportions of mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) in Iberian pigs (Ninoles et al., 2007). A decrease of sound velocity with increasing temperature was proven using differential scanning calorimetry (DSC), which is mainly assumed to be reasoned in different melting points of individual fatty acids (McClements and Povey, 1988; Ninoles et al., 2007). Combining the fact that differences in fatty acid composition influences the measured US parameters and the fact that backfat consists of 3 different layers with differing fatty acid compositions (Monziols, Bonneau, Davanel & Kouba, 2007; Daza et al., 2007) also indicates an influence of the thickness of individual backfat layers on sound velocity of the complete backfat. Close relations between the total backfat thickness and the composition of the fat layers (i.e. the total amount of fatty acids) were already stated in literature (Moody and Zobrisky, 1966; Davanel et al., 1999). Furthermore, relations of sound velocity with the amount of moisture and dry matter inside the layers have been assumed (Moody and Zobrisky, 1966; Fortin, 1986). However, by now no investigation targeted on US parameters of the individual backfat layers or the relationships to structural and compositional traits.

2.4 Relationships between ultrasound and biological tissue

Several studies have proven an influence of compositional differences (e.g. the amount of fat, protein or dry matter) on US measurements. Some of these findings have been summed earlier. However, beneath compositional differences there are also structural and methodical differences, which may influence the US measurements significantly and should therefore be considered carefully for every measurement.

2.4.1 Direction of sound propagation (anisotropy)

One of the major parameters affecting US measurements is a directional dependency of acoustic parameters caused by the structured alignment of muscle fibres. The relation

between muscle fibre direction and sound wave has been proven to affect attenuation and sound velocity; this is known as acoustic anisotropy (Miles and Fursey, 1977; Park et al., 1994b; Smith, 1996; Topp and O'Brien, 2000; Towa, Miller, Frizzell, Zachary & O'Brien, 2002). At bovine muscle, attenuation is highest measured with the sound field parallel to the fibre orientation with significantly lower values for measurements performed perpendicular (Roberjot, Laugier & Berger, 1994; Smith, 1996; Topp and O'Brien, 2000). As for attenuation, Smith (1996) also stated significantly higher sound velocity values at fibres measured parallel to the sound field compared to perpendicular ones.

A large variety of measurements were performed at different animals, muscles and under a wide range of specific conditions. However, due to a higher repeatability and comparability most of them were either performed parallel or perpendicular to muscle fibre direction. Only few investigations also targeted conditions comparable to hot carcasses. Mörlein (2005) performed measurements on hot porcine carcasses (45 min p.m.) and at the cold shear muscle (24 h p.m.) and found a highly significant influence of the measurement direction not only on sound velocity and attenuation but also on a wide range of spectral parameters. Higher backscatter values for example have been shown for measurements performed parallel to the muscle fibre direction. Furthermore, Mörlein (2005) stated a higher attenuation for muscles measured perpendicular which is contrary to earlier findings (Smith, 1996). Such differences could partially be explained by the measurement method (e.g. hot carcass / *in vitro*) and used US device (e.g. frequency). Furthermore, interactions between the anisotropy and further effects (e.g. temperature, maturation, rigor mortis) have to be considered as well.

2.4.2 Muscle structure

Beneath the acoustic anisotropy, both the contraction state and the intactness of the muscle fibres have also been mentioned to influence the acoustic properties. Smith (1996) stated higher attenuation and backscatter values on intact compared to homogenized tissue. In addition, attenuation and backscatter values have been shown to be higher in contracted compared to relaxed frog muscle fibres measured on a center frequency of 5 MHz (Glueck, Mottley, Sobel, Miller & Pérez, 1985). While the intactness of the fibres is of no practical relevance for early post mortem measurements, care has to be taken to avoid measurements during muscle contraction periods which are not uncommon early post mortem.

In addition, diameter and shape of muscle fibres and bundles are assumed to influence attenuation and backscatter values. In rat skeletal muscle the myofibres have been shown to be the most effective scatterers compared to myofibrils or even myofibre bundles (Topp and O'Brien, 2000). A relationship between the frequency dependence of the backscatter and the effective scatter diameter has also been stated for ocular tissue (Lizzi et al., 1997). Negative correlations were found between the sarcomere length and attenuation or sound velocity values at bovine muscle using scanning laser acoustic microscopy at 100 MHz for both, parallel and perpendicular measurement (Smith, 1996). However, comparable relationships may not be relevant for measurements performed at lower frequencies.

2.4.3 Temperature

A large influence of the temperature on acoustic parameters was stated for a wide variety of biological tissues. For muscle, an increasing temperature was found to be accompanied by a decrease of attenuation independent from muscle fibre direction (Smith, 1996). However, these relationships are not linear as the decrease from 4 °C to 20 °C is much larger than from 20 °C to 37 °C. These results were confirmed by further investigations using either ultrasound or shear force measurements at different temperatures (Sapin-de Brosses, Gennisson, Pernot, Fink & Tanter, 2010) while earlier studies also found comparable relationships for backscatter parameters (Abou El Karam, Buquet, Berge & Culioli, 1997).

High relationships with temperature were also found for sound velocity values. A nearly linear increase from 4 °C to 37 °C was stated for bovine muscle (Smith, 1996). This may partly be explained by the high amount of water inside the muscle were the sound velocity has been shown to increase with temperature (Del Grosso and Mader, 1972; McClements and Povey, 1992). However, in fat tissue the relationship is contrary, and the sound velocity constantly decreases with increasing temperature (Benedito, Carcel, Rossello & Mulet, 2001). At temperatures about approximately 24 °C, the sound velocity of fat is more or less equal compared to water (Miles and Fursey, 1977) and both tissues may not be differentiated by each other via time of flight measurement (Benedito, Carcel, Rossello & Mulet, 2001). Thus, samples with a large amount of fat may perform different in terms of sound velocity and increasing temperature compared to lean samples. To avoid this critical point, measurements should be performed at higher (about 37 °C) or lower (about 4 °C) temperatures to ensure detection of small differences.

These findings are not only true for muscle samples but are also affecting the backfat layers passed by the ultrasound signal. As for the muscle, the sound velocity is influenced not only by the amount of water but also by the chosen temperature as the melting of individual fatty acids may affect the sound velocity. Oleic acid for example, representing about 40 % of all fatty acids in the backfat layer has been stated to melt at about 5 to 15 °C (Cedeno, Prieto, Espina & García, 2001). The reduction of the solid-to-liquid ratio due to the melting of fatty acids with increasing temperature has been proven to reduce sound velocity using differential scanning calorimetry (McClements and Povey, 1992; Ninoles et al., 2007). Lower solid-to-liquid ratios in oils and fats have also been shown to result in lower sound velocity values (McClements and Povey, 1988). The melting point can be defined by the amount of carbon atoms and double bonds. The higher the number of carbon atoms and double bonds in fat, the lower the melting point and thus also sound velocity (Ninoles et al., 2007). However, most of the dominant fatty acids occurring in mammalian backfat melt in a temperature range between 0 to 20 °C. Therefore, differences in sound velocity which are dominant in lower temperature ranges may be negligible at hot-carcass conditions.

3 Objectives of the investigation

With regard to the objectives of this study, the published investigations allow the following conclusion:

- Multiple investigations stated an influence of structural (e.g. muscle fibre) and compositional (e.g. fat, water) parameters on meat quality even if the relationships are not always consistent.
- Different measurements have been shown to be able to analyse and determine the intramuscular fat content. However, none of these methods allows the prediction in a fast, cost-effective and non-destructive way.
- While the use of B-mode image analysis or sound velocity measurements resulted in good IMF prediction values at bovine muscle or hashed meat, investigations on porcine carcases or living pigs were less feasible.
- A wider range of information could be obtained analysing the backscattered ultrasound signals. First investigations on porcine muscle resulted in medium prediction errors and have proven the usability of spectral analysis.
- By now neither the used hardware nor the obtained accuracy predicting the intramuscular fat content in porcine muscle were satisfying for use in slaughter facilities.
- To obtain the highest accuracy for spectral analysis several correction functions are required for whom the published reference values are either not comparable to hot carcass conditions or no data has been published at all, by now.
- Multiple structural and compositional factors are known or anticipated to influence ultrasound measurements of muscle. So far, no investigation was performed completely covering a wide range of those factors and relating them to ultrasound parameters.

Therefore, the following targets could be stated for the investigation:

- Firstly, representative ultrasound data will be collected for muscle and backfat tissue. The obtained data will be related to structural and compositional traits to enable a prediction of factors influencing the ultrasound measurements.
- Secondly, the obtained muscle and backfat information will be implemented into the correction algorithms of a hand-held ultrasound device. The data should help to improve accuracy and reliability of the ultrasound measurements.
- Finally, a representative set of carcasses will be investigated with a hand-held ultrasound device using the improved correction algorithms. Spectral analysis of the obtained measurements will be used to investigate the ability of IMF prediction via ultrasound.

Paper I:

Koch, T.; Lakshmanan, S.; Brand, S.; Wicke, M.; Raum, K.; Mörlein, D.

Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle

Meat Science

Accepted: 3. December 2010

DOI: 10.1016/j.meatsci.2010.12.002

Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle

Koch, Tim *[a]; Lakshmanan, Sannachi *[b]; Brand, Sebastian [c]; Wicke, Michael [a]; Raum, Kay [b,d] and Mörlein, Daniel [a+]

[a]Department of Animal Sciences, University of Goettingen, Germany

[b]Julius Wolff Institute and Berlin-Brandenburg School for Regenerative Therapies, Charité-Universitätsmedizin, Berlin, Germany

[c]Fraunhofer Institute of Material Mechanics, Halle, Germany

[d]Department of Orthopedics, Martin Luther University of Halle-Wittenberg, Halle, Germany

* Both authors equally contributed to the study.

[+] corresponding author

Ultrasound velocity and attenuation of soft tissues have been widely investigated. However, few studies completely covered considerable variations of both, structure and composition. The aim of this study was to collect acoustic reference data of porcine *Longissimus* muscle and associate them with compositional traits. In addition, measurements were conducted on fresh, formalin fixed, and frozen-thawed samples to evaluate the effect of processing on ultrasound parameters and comparisons with earlier investigations. Measurement conditions (temperature, fibre orientation) were realised close to hanging carcasses conditions. Sound velocity ranged from 1617 ± 6 to 1622 ± 5 ms^{-1}, while attenuation mostly ranged from 1.0 ± 0.3 to 1.2 ± 0.3 dB MHz^{-1} cm^{-1}. Only formalin fixed samples showed significantly higher attenuation (2.2 ± 0.6 dB MHz^{-1} cm^{-1}). Highest correlations have been observed between intramuscular fat and attenuation (up to r = .7). The obtained results are anticipated to improve ultrasound based estimation of the intramuscular fat of pig muscle on intact carcasses.

Keywords: Ultrasound, spectral analysis, attenuation, sound velocity, longissimus, pork, muscle, fibre type, intramuscular fat, meat quality

1. Introduction

Pork is the most important meat in Germany with approximately 60 kg consumed per capita and year. Factors like pig breed, gender, feeding, ante mortem handling, and post mortem treatment have been shown to affect the final meat quality of the economically important loin *Longissimus* muscle (Rosenvold and Andersen, 2003). One of the most important meat quality traits is the intramuscular fat content (IMF) that is widely regarded to influence the sensory characteristics of pork loin (Fernandez, Monin, Talmant, Mourot & Lebret, 1999).

Many investigations were targeting non-invasive IMF estimation on intact pig carcasses. Infrared spectroscopy (Alomar, Gallo, Castaneda & Fuchslocher, 2003) and dual energy X-ray absorptiometry (Suster, Leury, Ostrowska, Butler, Kerton, Wark & Dunshea, 2003) have been shown to be reasonably correlated with fat content. However, these methods are either time consuming, destructive, or very cost intensive, and thereby not feasible to be used at slaughter.

Ultrasound (US) is regarded as a fast, cost effective and non-destructive method for tissue characterization. Several studies have indicated the potential of US for the prediction of bovine IMF or marbling score in muscle. For example, the pulse travel time, hereinafter referred to as “time-of flight” (TOF), of the transmitted US waves has been used to predict the fat content of raw meat mixtures or excised beef muscle (Park, Whittaker, Miller & Hale, 1994; Benedito, Carcel, Rossello & Mulet, 2001). However, this method can only be used ex-vivo. Analyses of grey-scale B-mode US images using different statistical parameters of grey value distribution, e.g. Fourier-based and histogram based parameters have been successfully applied to predict IMF in living steers (Hassen, Wilson, Amin, Rouse & Hays, 2001; Hassen, Wilson & Rouse, 2003). In porcine loin muscle, this image analysis was shown to be less feasible (Newcom, Baas & Lampe, 2002). This was suggested to be due to structural and compositional differences, such as the considerably wider range of IMF in bovine muscle (1.1 to 11.2 %) compared to the rather low IMF range in pork loin (0.3 to 3.6 %), typical for German or western European pig populations (Hassen et al., 2001; Link, 2007).

In contrast to grey-scale image analysis or time of flight measurements, ultrasound spectral analysis is suggested to provide more comprehensive information about tissue constitution (Lizzi, Feleppa, Alam & Deng, 2003). Thus, ultrasound spectral analysis is

widely investigated for enhanced tissue characterisation in medical research (Nair, Kuban, Obuchowski & Vince, 2001; Oelze, Zachary & O'Brien, 2002; Scheipers, Ermert, Sommerfeld, Garcia-Schürmann, Senge & Philippou, 2003; Gelse, Olk, Eichhorn, Swoboda, Schoene & Raum, 2010). In a previous study spectral analysis of backscatter signals obtained with a sophisticated medical ultrasound device was used to non-invasively estimate the intramuscular fat content of porcine loin muscle (Mörlein, Rosner, Brand, Jenderka & Wicke, 2005). Multivariate prediction of IMF with several spectral parameters (amplitudes and gradients) yielded average prediction errors of 0.36 % and demonstrated the feasibility of this technique even in the low IMF range. However, both the instrument and the prediction errors were not yet satisfying for industrial use at slaughter. A prerequisite for a reliable spectral analysis is the accurate correction of frequency dependent attenuation and refraction effects (caused by variations of attenuation and sound velocity) in skin, backfat and muscle tissues along the sound propagation path between transducer and the evaluated region of interest (ROI). Incorporation of these potential variations for individual measurements are anticipated to increase the robustness of the ultrasound based IMF prediction.

Acoustic attenuation and sound velocity of soft tissues have already been studied in different species and tissues including muscle (Chivers and Parry, 1978; Greenleaf, 1986; Topp and O'Brien, 2000). However, most of these investigations have been conducted either on differing species or on frozen or fixed samples (Miles and Fursey, 1977; Nassiri, Nicholas & Hill, 1979; Smith, 1996; Topp and O'Brien, 2000). To our knowledge, no representative set of data exist for native porcine fat and muscle tissues obtained under in-vivo or close-to in-vivo conditions. Tissue type, composition, but also preparation and measurement conditions are known to affect acoustic properties (Nassiri et al., 1979). For example, temperature has been reported to reversely affect ultrasound velocity in fat and muscle (Miles and Fursey, 1977; Park et al., 1994; Smith, 1996; Benedito et al., 2001). Moreover, the well structured alignment of muscle fibres causes a directional dependency of acoustic parameters, known as anisotropy (Topp and O'Brien, 2000; Towa et al., 2002). For muscle, attenuation and sound velocity were reported to be remarkably affected by the direction of the incident sound waves. In bovine muscle attenuation has been shown to be up to 50 % higher for measurements performed parallel to the fibre direction compared to measurements performed perpendicular to the fibres (Smith, 1996). Minor differences were found for sound velocity. Measurements performed parallel to the fibre direction were only 0.5 to 1 % higher compared to measurements performed perpendicular to the fibres (Smith, 1996; Topp and O'Brien, 2000).

To our knowledge, there is no ultrasound study of muscle that comprehensively considers the variability of histological parameters, e.g. muscle fibre size and types, chemical composition, physical meat quality parameters (e.g. drip loss), and sample preparation with respect to variations of IMF and acoustic properties. Some studies investigated relations between ultrasound and IMF content (Park et al., 1994; Newcom et al., 2002) without considering physical properties such as water holding capacity. Other investigations mainly referred to anisotropy effects (Smith, 1996; Topp and O'Brien, 2000) or the relationships between the fibre type composition of the muscle and the pH, drip loss and the post mortem metabolic rate and thus the meat quality (Ryu and Kim, 2005). Furthermore, most of the previous ultrasound investigations have been performed either on fresh, fixed, or frozen muscle samples resulting in a lack of comparability between the investigations.

In this study, acoustic attenuation and sound velocity reference data of porcine *m. longissimus* were collected *in-vitro* in a set of pig carcasses resembling a variation of carcass properties typically found in German slaughter facilities. The variations of these acoustic properties were compared to those of IMF and compositional, physical and structural traits. Temperature and sound propagation direction relative to the muscle fibre direction were carefully adjusted to ensure close-to typical in-vivo-conditions, i.e. measurements on hot carcasses 45 min p.m.. At that time, ultrasound readings are usually applied to predict the carcass lean meat percentage.

Since ultrasound experiments under laboratory conditions can usually not be conducted within this time frame, we aimed at analyzing the effects of tissue ageing and storage (either by freezing or formalin fixation) on the measured acoustic properties.

Moreover, we hypothesized that variations of speed of sound and attenuation are not only linked to variations of IMF, but also to other tissue variations, that may potentially have an effect on the acoustic properties. The establishment of these relations are anticipated to improve sound field correction and subsequent spectral rf data processing techniques needed for ultrasound based IMF predictions *in vivo*.

2. Materials and methods

2.1. Animals, meat quality parameters and chemical determinations

A variation of meat properties representative for German slaughter facilities was obtained by selection of pig carcass sides from a commercial abattoir (left half each). Care was taken to select a wide variety of carcass weight and lean meat percentage (LMP) with 3 levels for both parameters, resulting in a total of n = 27 carcasses. The animals were stunned

with CO_2, exsanguinated and scalded at approximately 62 °C. After evisceration, muscle (MW) and backfat width (BW) were determined with a carcass grading probe (Fat-o-Meat'er, Carometec, Denmark) between $2^{nd}/3^{rd}$ last rib. Meat quality indicators, i.e. pH value (Portamess 913, SE 104 glass type probe; Knick GmbH, Berlin, Germany) and electrical conductivity (EC; LF-Star; Matthäus, Nobitz, Germany) were recorded 45 min p.m. on the same location. The pH meter was calibrated using 2 buffer solutions at pH 4 and 7 (Carl Roth GmbH, Karlsruhe, Germany) and adjusted for temperature. Approximately 24 h p.m., after chilling of the carcasses over night, three adjacent chops were excised from the *m. longissimus* at the $3^{rd}/4^{th}$, $2^{nd}/3^{rd}$, and $1^{st}/2^{nd}$ last rib for subsequent laboratory analyses.

Meat colour values (CIE-L*,a*,b*) were recorded 24 h p.m. in triplicate at the chop surface after 10 min blooming time (CR-300 chromameter (illumination: D65, closed cone); Minolta, Langenhagen, Germany). Calibration was performed using a calibration plate (CR-A43; Minolta, Langenhagen, Germany). Drip loss (DL) was measured using the EZ drip-loss method (Christensen, 2003). Briefly, two cylindrical muscle samples (about 10 g each) per chop were obtained 24 h p.m. from the $2^{nd}/3^{rd}$ last rib and stored in plastic containers for 48 h at 4 °C. The samples were weighted before and after storage and the difference was calculated. Drip loss is given as percentage amount of the lost weight compared to the weight of the sample before storage.

Samples for ultrasound data acquisitions were prepared approximately 24 h p.m. and were either measured directly after dissection or after subsequent preparation steps (2.2).

Chemical analyses to determine intramuscular fat content (IMF), dry matter (DM), and protein (all related to fresh matter) were performed on homogenised samples from the $2^{nd}/3^{rd}$ last rib after removal of the subcutaneous fat. The homogenization was done using a Grindomix GM200 (Retsch GmbH; Haan, Germany) homogenizer with 6000 rpm for 30 seconds. IMF was determined with petroleum ether using a Soxtherm-apparatus after HCl pre-treatment according to German Food Legislation (LFGB, 2005). The determination of dry matter was performed on small samples (~5 g) that were dried in sea sand at 103°C until equilibrium weight was reached. Protein content was determined automatically after oxidative digestion (VarioMax; Elementar GmbH, Hanau, Germany).

Histology samples were obtained 24 h p.m. from samples of the $2^{nd}/3^{rd}$ last rib. The samples were snap-frozen in liquid nitrogen and stored at -60 °C until use. A modified ATPase/NADH-TR staining (Horák, 1983) was performed on 10 µm cross cryosections perpendicular to fibre direction (CM1900; Leica Microsystems GmbH; Wetzlar, Germany). For determination of fibre type proportions, slow-twitch oxidative (STO), fast-twitch

glycolytic (FTG), and fast-twitch oxidative (FTO) fibres were counted and the fibre diameters were determined manually. At least 2 fields of view and 300 fibres per sample were examined on digital light micrographs (100 x magnification; NIS-Elements; Nikon GmbH, Düsseldorf, Germany).

2.2. Ultrasound data acquisition

A custom-made scanning acoustic microscope (SAM) was used for data acquisition (Raum, 2008). The SAM consisted of a pulser-receiver unit (5900PR, Panametrics, Waltham, USA), a 10-MHz central frequency transducer (V311, Panametrics, Waltham, USA), a 3-axis scan stage including a motion controller (Micos, Eschbach, Germany) and a computer containing a 12-bit analogue-to-digital converter (CS12400, Gage Applied Technologies, Lachine, Canada). The received echo signals were digitized at 50 MS/s and the input range of the analogue-digital converter was set to ±500 mV.

Prior to ultrasonic data acquisition, 4 subsamples with an edge length of about 15 mm were prepared from 2 excised chops. Care was taken to ensure that sample orientation was similar to the measurement on the intact carcass, i.e. that the muscle fibre orientation was 30° to 45° relative to the sound propagation direction (Fig. 1). Four groups of samples were evaluated for each carcass. The first sample ($2^{nd}/3^{rd}$ last rib) was measured 24 h p.m. (group A: native, 24 h p.m.) while a second sample ($1^{st}/2^{nd}$ last rib) was kept at 4°C for another 24 h and was measured 48 h p.m. (group B: native, 48 h p.m.). 24 h p.m., two adjacent samples from the $3^{rd}/4^{th}$ last rib were either fixed in 5 % formalin (group C: formalin fixed) or stored at -60°C until measurement (group D: frozen-thawed). Ultrasound measurements of fixed and frozen-thawed samples were performed within 4 months after slaughter. Each sample was placed in a custom made multi-chamber-holder (Fig. 2). This chamber holder allowed the acquisition of pulse echoes from a plane steel reflector with and without sample, as well as tight attachment of the sample without compression. The scans were performed with a step width of 0.4 by 1 mm, resulting in approximately 150 radio frequency (rf) lines per chamber. For coupling of the acoustic waves the sample and the transducer were submerged in degassed 0.9 % phosphate-buffered-saline (PBS). The PBS solution was prepared using 9.55 g/L Dulbeccos powder (AppliChem; Darmstadt, Germany), consisting mainly of NaCl (80%) and Na_2HPO_4 (15%). The concentration of the buffered solution corresponds to slightly less than 0.15 M saline and simulates close-to physiological conditions. This concentration has been chosen in accordance to earlier investigations on porcine muscle that reported no

influence on intramuscular NaCl level or fibre diameter due to swelling for similar sample sizes (4 cm^3) and salt concentrations (Böcker, Ofstad, Bertram, Egelandsdal & Kohler, 2006).

The ultrasound velocity of the PBS (v_{PBS}) was measured prior to every sample measurement and the specific velocity values were included in further calculations. The temperature was kept constant at 38.0° ± 0.1°C. Prior to each measurement the samples were allowed to equilibrate in PBS. Formalin fixed samples were washed in PBS for 24 h prior to the ultrasound measurements.

2.3. Ultrasound data analysis

Ultrasound velocity and acoustic attenuation were analysed using a custom made MATLAB (The Mathworks, Natick, USA) based software package. Briefly, this software allows a semi-automatic detection of the front and backside reflections within the sample area and the detection of the steel plate reflection in the adjacent reference chambers (Figs. 2 and 3). From these echoes the travel times t_i were determined from the location of the maximum of the Hilbert-transformed envelope signal (Lakshmanan, Bodi & Raum, 2007). Thickness and sound velocity were calculated from travel time differences with and without the sample (see Fig. 3) using a substitution method (Strelitzki, Clarke & Evans, 1996):

$$v_{sample} = \frac{2d}{(t_3 - t_2)} = \frac{v_{PBS}(t_1 - t_2)}{(t_3 - t_2)}. \quad (1)$$

Acoustic attenuation was estimated by comparing the logarithmic power spectra $S_{dB}(f)$ of pulses obtained from the tissue-backplate reflector to the spectra measured in the reference chambers (Laugier, 2008):

$$S_{dB}(f) = 10\log_{10}\left(\left|FFT\left(V(\Delta t)\right)\right|^2\right), \quad (2)$$

whereas FFT is the fast Fourier transformation of the rf pulse echo signal $V(\Delta t)$ within the time interval Δt.

The difference spectra were normalized to the sample thickness and a linear fit was performed within the frequency band for which sufficient signal amplitudes were obtained in the attenuated signals (3 to 6 MHz). The slope of the linear fit provided the attenuation coefficient in dB MHz^{-1} cm^{-1}.

Prior to signal analysis, a region of interest (ROI) in the central part of the chamber was manually selected resulting in approximately 100 rf-lines. For each rf-signal the local sound velocity was estimated. Individual rf pulse-echo signals with a discrepancy of more

than 1.5 times of the standard deviation of the mean velocity estimate were excluded from further analyses. These outliers were mainly caused by a partial detachment of the sample from the backplate. Finally, about 75 rf-signals per sample were used for estimating speed of sound and frequency dependent attenuation. Each data set was analyzed by two independent trained users to estimate the effects in terms of software handling. The mean differences between both users were less than 0.1 % for sound velocity and less than 2.5 % for attenuation. Therefore, the data processing was considered not to be affected by insufficient handling of the software if performed by trained users. Subsequently, mean values of both users were used for further statistical analyses.

2.4. Statistical analyses

The statistical analysis was performed with SAS 9.1 (SAS Institute, Cary, USA), STATISTICA 7.1 (StatSoft, Tulsa, USA) and The Unscrambler 9.2 (CAMO ASA, Oslo, Norway). To evaluate the impact of the IMF class, ageing, and sample preparation method (24 h, 48 h, formalin-fixed, frozen-thawed) all samples were first checked for normal distribution, performing a Shapiro-Wilks test. Three IMF groups (LOW, MID, HIGH) were selected according to mean IMF ± ½ standard deviation. Thereafter, a two-way analysis of variance (ANOVA) was performed with ageing time (24 h, 48 h) and IMF as fixed effects to evaluate the combined effect of both IMF group and ageing on ultrasound parameters. A second two-way ANOVA was performed with preparation type (native 24 h, formalin-fixed, frozen-thawed) and IMF as fixed effects to evaluate influence on ultrasound parameters. Thereafter, one-way ANOVAs were applied to investigate the influence of the IMF groups on the native samples.

Linear correlations between acoustic, structural and compositional parameters were computed as Pearson product-moment correlations.

Multiple linear regression (MLR) was performed estimating the influence of all compositional and structural parameters on attenuation and sound velocity using STATISTICA 7.1 and Unscrambler 9.2. In all cases full-cross validation was used to verify the results. Thus, every sample was left out once to test the model constructed out of the remaining 26 samples. Variables were included into the model if the required level of significance reached $p < .05$.

All statistical results were considered significant for p values smaller than .05.

3. Results

3.1. Carcass and muscle characteristics

Descriptive statistics of carcass features are given in Table 1. As selected by the ranges of LMP and carcass weight, IMF content was widely spread (Fig. 4). The relative variations of drip and thawing losses were in the same range as that of IMF. The smallest variations were found for pH 45 min p.m. and the relative amounts of protein and dry matter. For histological traits, the average muscle fibre diameter of FTG fibres was significantly higher compared to those of FTO and STO fibres (F = 39.8). The IMF content was correlated with muscle thickness (r = -.57), protein content (r = -.48) and the relative amount of FTG fibres (r = -.47) (data not shown).

3.2. Ultrasound parameters

Ultrasound velocity and attenuation values (median, upper and lower quartile) of muscle samples with respect to sample preparation are shown in Figs. 5 and 6. The mean and standard deviations in group A (native, 24 h p.m.) were 1620.5 ± 4.6 ms^{-1} for SOS and 1. 02 ± 0.26 dB MHz^{-1} cm^{-1} for attenuation. Refrigerated storage for 24 h did not significantly alter the acoustic properties of the samples within the evaluated time frame from 24 h to 48 h p.m.. Freezing or fixation in formalin remarkably increased the variance of the sound velocity values. However, the measured differences did not reach the significance level. In accordance, two-way ANOVA did not reveal any significant sound velocity differences for either IMF-group or preparation type.

The attenuation of 2.23 ± 0.60 dB MHz^{-1} cm^{-1} in group C (formalin fixed samples) was significantly higher compared to those measured in groups A, B and D (F = 53.56). Moreover, it should be noted that the standard deviations of both estimated parameters were considerably higher in group C compared to those of groups A and B (Figs. 5 and 6). Two-way ANOVA confirmed the tissue preparation effect (F = 74.4), but also revealed significant differences between the IMF groups (F = 21.3).

3.3. Relationships of ultrasound parameters with carcass and muscle characteristics

The Pearson correlation coefficients of the associations between ultrasound parameters and selected meat quality traits are summarized in Table 2. Most obviously, acoustic attenuation was correlated with IMF, regardless of the tissue preparation type (-.4 to -.7). In accordance to that, significant differences could be stated between the attenuation of the IMF classes (native 24 h and 48 h) with highest values in the HIGH class as shown in Table 3.

After pooling both native classes a two-way ANOVA with ageing and IMF group as fixed effects revealed significant impacts of both parameters (IMF: F = 11.7; ageing: F = 4.1).

Relationships between the attenuation of native samples and histological traits (data not shown) have been found for the amount of FTG (r = -.31 to -.50) and STO fibres (r = .29 to .43). No comparable finding could be stated for sound velocity.

A MLR model for sound velocity could only be stated for group A (Eq. 3). Both, IMF and protein content showed significant correlations to sound velocity. Due to cross-correlations between IMF and protein (r = -.48), only the IMF remained as a predictive variable in the model (validation R^2 = .13; RMSE = 4.33 ms^{-1}).

$$v_{sample}(24\ h\ p.m.) = 1625.6\ ms^{-1} - 3.94\ ms^{-1} \cdot IMF \quad (3)$$

Slightly better results could be obtained modelling the attenuation. For group A only the IMF value was used as predictive variable (Eq. 4; R^2 = .34; RMSE = 0.21 dB MHz^{-1} cm^{-1}) due to auto-correlations between IMF and drip loss (r = -.38).

$$\alpha^{A}_{sample}(24\ h\ p.m.) = 0.651\ dBMHz^{-1}cm^{-1} + 0.290 dBMHz^{-1}cm^{-1} \cdot IMF \quad (4)$$

Group B could be explained by a combination of IMF, pH and drip loss (Eq. 5) as significant variables (R^2 = .45; RMSE = 0.25 dB MHz^{-1} cm^{-1}).

$$\alpha^{B}_{sample}(48\ h\ p.m.) = \left(-5.952 + 0.446 \cdot IMF + 0.972 \cdot pH + 0.057 \cdot DL\right) dBMHz^{-1}cm^{-1} \quad (5)$$

Less clear relationships between IMF and sound velocity (r = .0 to -.5) compared to attenuation can also be seen comparing the IMF classes against each other (Table 3). Significant differences could only be observed between the LOW and the HIGH class of group A (F = 2.4), while no differences in sound velocity occurred for group B (F = 0.3). Two-way ANOVA with ageing and IMF group as fixed effects again did not reveal any significant relationships.

4. Discussion

4.1. Carcass and muscle characteristics

Variations and relationships of carcass traits, physical and chemical muscle characteristics (e.g. IMF, DL, pH) are in good agreement with earlier investigations at German pig populations (Laube, Henning, Brandt, Kallweit & Glodek, 2000; Fiedler, Dietl, Rehfeldt, Wegner & Ender, 2004; Mörlein, Link, Werner & Wicke, 2007). As expected, IMF was slightly increased with backfat thickness and, subsequently decreased with higher muscle

thickness (Mörlein et al., 2005). While the muscle fibre type composition was similar, fibre diameters were slightly higher compared to previous studies (Miller, Garwood & Judge, 1975; Fiedler et al., 2004). Such differences are most probably due to the pigs breed type and age at slaughter, which we did not intend to fully control in our study.

In accordance to the given characteristics and relationships the selected carcasses can be considered to be representative for German pig populations with regard to carcass traits, structure and composition.

4.2. Ultrasound parameters

The obtained sound velocity estimates of *longissimus* muscle in the present study are mostly in accordance with earlier findings in mammalian skeletal muscle (Chivers and Parry, 1978). Sound velocities between 1590 – 1610 ms^{-1} were found for bovine frozen-thawed skeletal muscle measured at 37 °C (Miles and Fursey, 1974) while the frozen-thawed samples in our investigation reached values from 1602 – 1626 ms^{-1}. Measurements in fresh bovine muscle have been stated with higher velocity values (~1650 ms^{-1}) at the same temperature (Smith, 1996). Slight differences however may be explained with both, the investigated species and differences in muscle fibre orientation.

An increased loss of meat juice up to 10 % of fresh weight in muscle samples due to freeze-thawing was stated earlier (Miles and Fursey, 1977; Ngapo, Babare, Reynolds & Mawson, 1999) indicating a potentially higher sound velocity due to the decreased amount of moisture in the freeze-thawed samples (Miles and Fursey, 1977). No comparable finding however, could be stated for our investigation. The loss of water was probably compensated by submerging the samples in PBS for acoustic coupling, by which the muscle samples were enabled to rehydrate to some extend. To avoid swelling of the sample above the level of rehydration the saline concentration inside the PBS was chosen at a low level of molarity. Offer and Trinick (1983) noted that concentrations of about 0.4 M saline / phosphate solution would be required to induce swelling. Furthermore, a minor influence on the structure of actin and myosin was found for porcine muscle samples (500 mg) in a 10 g/L NaCl solution after 3 h using differential scanning calorimetry (Graiver, Pinotti, Califano & Zaritzky, 2006). Thus, rehydration in 0.9 % PBS solution should not be expected to exceed the substitution of lost water during the comparably short periods of rehydration used during our investigation.

Similar to frozen samples, fixation with formalin did not significantly change the ultrasound velocity within this study. A slight increase of 12 ms^{-1} after fixation has been stated for myocardial tissue (Baldwin, Yang, Marutyan, Wallace, Holland & Miller, 2005).

However, the same investigation also stated a decrease in sound velocity of about 10 ms^{-1} after holding the formaldehyde fixed samples inside a water bath for about 3 h prior to measurement. This is comparable to our protocol as samples were kept inside the PBS for about 12 hours to replace the formaldehyde prior to the ultrasound data acquisition. According to these results the sound velocity of muscle can be assumed to be independent of freezing / fixation if the samples are washed and enabled to rehydrate prior to the measurements.

Attenuation of native muscle samples was found to be about 1.1 dB MHz^{-1} cm^{-1} in the present study. Comparable values of 1.3 to 2.9 dB MHz^{-1} cm^{-1} (parallel) and 0.55 to 1.1 dB MHz^{-1} cm^{-1} (perpendicular) have been observed for native bovine skeletal muscle measured at 20°C (Nassiri et al., 1979). Measurements are usually performed with incident sound waves strictly parallel and/or perpendicular to the muscle fibre direction and considerable differences of acoustic attenuation were reported with respect to the fibre orientation also referred to as anisotropy (Nassiri et al., 1979; Topp and O'Brien, 2000). In the present study the fibre direction was not chosen strictly in one orientation (Fig. 1) as the intention was to obtain data at close-to in-vivo conditions. Therefore, more precise comparisons with literature values are rendered impossible. Furthermore, a temperature dependency of attenuation has to be taken into account. For example, a range from 1.26 to 1.97 dB MHz^{-1} cm^{-1} between 0 °C and 40 °C with lowest values at 20 °C has been reported for bovine muscle (Shore, Woods & Miles, 1986) without giving any specifications of anatomic origin or compositional properties.

As for sound velocity no influence of either ageing or freeze-thawing on attenuation could be stated. A considerable increase of about 100 % however, was found for the formaldehyde-fixed samples. Comparable findings with an attenuation increase of about 50 % for fixed skeletal muscle samples has already been reported for beef (Nassiri et al., 1979). This increase is suggested to be due to (expected) cross-links, e.g. the association of side groups of certain amino acids of adjacent proteins (Bamber, Hill, King & Dunn, 1979).

4.3. Relationships of ultrasound parameters with meat quality traits

Within this study ultrasound parameter estimates were related to a set of structural and compositional traits of the muscle samples under investigation. Sound velocity in fresh beef muscle measured along the fibre axis has been reported to be negatively correlated with IMF ($r = -.82$) (Park et al., 1994). This could partially be confirmed by our work for native muscle samples measured 24 h p.m. ($r = -.50$). Relationships were not as close for the samples

analyzed after 48 h the formalin fixed and for the frozen thawed samples. The higher correlations mentioned by Park et al. (1994) may be favoured by the tremendously higher IMF range in bovine muscle (~ 2 – 14 %). As for correlations, comparing sound velocity between LOW and HIGH IMF classes revealed small but significant differences for group A being about 0.5 % smaller in the HIGH group. Comparably small differences have been published for hashed meat where the 0 % fat sample showed a less than 2 % higher sound velocity compared to the sample with 30 % fat at 25 °C (Benedito et al., 2001). Differences in the correlation coefficient between IMF and US parameters of samples A and B may partially be explained by variable composition even between adjacent samples within muscle, especially as no influence of ageing could be stated. Intramuscular fat, also referred to as marbling, is not evenly distributed within the pork loin and the chemically determined amount of IMF may therefore not be fully representative for small samples.

Sound velocity was also reported to be negatively correlated with the pH (Ayadi et al., 2007), which could not be confirmed by our findings. However, these measurements were performed on native beef muscle 1 hr p.m. while our US measurements were performed 24 h p.m. at the earliest. Even though a higher loss of water for low pH values can be expected and has been confirmed by our results (not shown), the rehydration due to PBS is suggested to partially compensate for this effect. An influence of different pH values on the amount of rehydration and swelling (and thus potentially also on US parameters) as stated for higher saline concentrations (Böcker et al., 2006) can be excluded in our study. Böcker (2006) stated no influence of the pH values on swelling at low salt concentrations (0.9 %) after 8 days in porcine muscle with a pH range by far exceeding our investigation (pH24: 5.6 – 6.4).

In terms of ultrasound correction algorithms, the obtained relationships between compositional parameters and sound velocity are promising. Besides IMF the given sound velocity values can be considered to be mostly independent from the compositional parameters investigated in this study. Correlations of sound velocity with e.g. protein content or histological traits (not shown) are always minor and never exceed r = .41 for native samples. Furthermore, auto-correlations between compositional parameters (e.g. protein) and IMF may cause correlations with sound velocity. Overall, the low coefficient of variation (~ 0.3 %) of sound velocity indicates only a minor effect of muscle structure and composition. Therefore, while sound velocity seems to be unsuitable for IMF determination in porcine muscle as stated for bovine muscle or hashed meat, the obtained dataset can be used as reference value. Moreover, independency of compositional parameters, at least for the

investigated range, allows the use of the obtained velocity data as reference values without any required corrections for e.g. muscle size or pH.

Attenuation was found to be moderately correlated with IMF (r = .4 to .7; Table 2) for all investigated treatments. These findings are in accordance with previous investigations in porcine muscle stating an about 50 % higher attenuation in groups with high IMF level (Mörlein et al., 2005). Relationships between the amount of IMF and the attenuation have also been published for beef muscle as a decreasing attenuation with increasing water content has been (Smith, 1996). Minor positive relationships with dry matter obtained during our investigations confirm these findings. Moderate to strong positive correlations have been stated between attenuation and pH (Ayadi, Culioli & Abouelkaram, 2007). Accordingly, attenuation showed a negative relationship with drip loss and positive ones with pH and EC (data not shown) in the present study. This is presumably caused by a faster protein and cell denaturation at lower pH values. Again, rehydration of samples due to the measurement set up in PBS may have counterbalanced some of the relationships.

Earlier investigations mentioned relationships between the acoustic properties (e.g. attenuation) and orientation and size of the muscle fibres (Topp and O'Brien, 2000; Lizzi et al., 2003) which can not be confirmed by our investigation. This may however be reasoned in slight differences of the muscle fibre orientation between individual carcasses. Even though the overall direction of the muscle fibres is comparable for all carcasses, differences in the orientation and therefore the angle of insonification (mostly between 30° and 45°) are inevitable. Thus, minor angle deviations may have affected the acoustic parameters in our study.

The slight relationships between the amount of FTG (up to r = -.47) and STO fibres and attenuation found during this investigation reflect the correlations between IMF and attenuation as the STO fibres are related to higher amounts of IMF (Leseigneur-Meynier and Gandemer, 1991).

Contrary to sound velocity, the attenuation seems to be more influenced by the investigated parameters with the IMF again being the most important one. The measured coefficient of variation ranges between 25 and 30 %, which is about 100 times higher than that for sound velocity. Therefore, the usability of attenuation predicting the IMF content in loin muscle as already stated in literature (Smith, 1996; Mörlein et al., 2005) can be proven by our results. As a further advantage, the attenuation was mostly unaffected by compositional

parameters (including histological differences) besides IMF. Only pH, drip loss, and the amount of FTG fibres showed additional minor influences on attenuation of the native samples. All of these relationships however are rather small compared to those between attenuation and IMF and can partially be explained by correlations with the IMF causing an auto-correlation between attenuation and e.g. the amount of FTG fibres. Therefore, the majority of the investigated parameters can be considered to either not influence the attenuation of the loin muscle or to be already described by the influence of the IMF. These findings will help to classify ultrasound measurements intending to predict compositional traits of muscle.

5. Conclusions and implications

For the first time, attenuation and sound velocity of native, fixed and frozen-thawed samples of porcine *longissimus* muscle of 27 pig carcasses, representative for German pig populations were estimated under laboratory conditions and related to physical and chemical properties. Significant differences between sample treatments were found for attenuation which was highest for formalin fixed samples compared to native or frozen-thawed samples. No differences were found for the velocity values between treatments. Among the ultrasound parameters attenuation was related to compositional parameters with intramuscular fat being the by far most important one. Less distinct relationships were found between the attenuation, pH and drip loss being important meat quality traits. Sound velocity showed only a slight negative correlation with IMF, being less than 5 ms^{-1} lower for samples in the HIGH IMF group.

Correlations were not always consistent between sample treatment which is assumed to be caused by post mortem muscle property changes and by intraindividual differences between adjacent samples. Rehydration and muscle swelling in PBS due to the measurement set up and relationships to the pH value has been discussed.

These data will help to elucidate how ultrasound parameters are influenced by structural and chemical composition of muscle tissue. The effects of sample freeze-thawing and fixation with formaldehyde have been quantified, which are useful for the comparison of literature data and for further investigations, where measurements of native samples are impossible. Moreover, due to minor variances of the sound velocity with differing composition, the IMF dependencies of attenuation and velocity can be directly utilized for the optimization of refraction and attenuation correction algorithms. The implementation of the observed relations in the data processing rather than the estimation of attenuation and sound

velocity from the measurement at the carcass has the advantage that artifacts, e.g. caused be imperfect coupling can be avoided. By this, we anticipate an improvement of the accuracy of ultrasound based pig carcass grading devices dedicated to the non-invasive estimation of intramuscular fat content.

Acknowledgements

This work was financially supported by the German Research Council (DFG; grants Mo 1746/1 and Ra 1380/3), PRO INNO (grant KF0380401LF6) and SFK Technology GmbH.

Reference List

Alomar, D., Gallo, C., Castaneda, M. and Fuchslocher, R. (2003). Chemical and discriminant analysis of bovine meat by near infrared reflectance spectroscopy (NIRS). *Meat Science*, 63, 441-450.

Ayadi, A., Culioli, J. and Abouelkaram, S. (2007). Sonoelasticity to monitor mechanical changes during rigor and ageing. *Meat Science*, 76, 321-326.

Baldwin, S. L., Yang, M., Marutyan, K. R., Wallace, K. D., Holland, M. R. and Miller, J. G. (2005). Measurements of the anisotropy of ultrasonic velocity in freshly excised and formalin-fixed myocardial tissue. *J.Acoust.Soc.Am.*, 118, 505-513.

Bamber, J. C., Hill, C. R., King, J. A. and Dunn, F. (1979). Ultrasonic propagation through fixed and unfixed tissues. *Ultras.in Med & Biol*, 5, 159-165.

Benedito, J., Carcel, J. A., Rossello, C. and Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 57, 365-370.

Böcker, U., Ofstad, R., Bertram, H. C., Egelandsdal, B. and Kohler, A. (2006). Salt-induced Changes in Pork Myofibrillar Tissue Investigated by FT-IR Microspectroscopy and Ligh Microscopy. *Journal of Agricultural and food chemistry*, 54, 6733-6740.

Chivers, R. C. and Parry, R. J. (1978). Ultrasonic velocity and attenuation in mammalian tissues. *J.Acoust.Soc.Am.*, 63, 940-953.

Christensen, L. B. (2003). Drip loss sampling in porcine *m. longissimus dorsi*. *Meat Science*, 63, 469-477.

Fernandez, X., Monin, G., Talmant, A., Mourot, J. and Lebret, B. (1999). Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of *M. longissimus lumborum*. *Meat Science*, 53, 67-72.

Fiedler, I., Dietl, G., Rehfeldt, C., Wegner, J. and Ender, K. (2004). Muscle fibre traits as additional selection criteria for muscle growth and meat quality in pigs - results of a simulated selection. *J.Anim.Breed.Genet.*, 121, 331-344.

Gelse, K., Olk, A., Eichhorn, S., Swoboda, B., Schoene, M. and Raum, K. (2010). Quantitative ultrasound biomicroscopy for the analysis of healthy and repair cartilage tissues. *Europ.Cells and Materials*, 19, 58-71.

Graiver, N., Pinotti, A., Califano, A. and Zaritzky, N. (2006). Diffusion of sodium chloride in pork tissue. *Journal of Food Engineering*, 77, 910-918.

Greenleaf, J. F. (1986). *Tissue characterization with ultrasound (Volume I)*. Florida: CRC Press, Inc.

Hassen, A., Wilson, D. E., Amin, V. R., Rouse, G. H. and Hays, C. L. (2001). Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *Journal of Animal Science*, 79, 11-18.

Hassen, A., Wilson, D. E. and Rouse, G. H. (2003). Estimation of genetic parameters for ultrasound-predicted percentage of intramuscular fat in Angus cattle using random regression models. *Journal of Animal Science*, 81, 35-45.

Horák, V. (1983). A Successive Histochemical Staining for Succinate Dehydrogenase and "Reversed"-ATPase in a single Section for the Skeletal Muscle Fibre Typing. *Histochemistry*, 78, 545-553.

Lakshmanan, S., Bodi, A. and Raum, K. (2007). Assessment of anisotropic tissue elasticity of cortical bone from high-resolution. angular acoustic measurements. *IEEE Trans.Ultrason.Ferroelectr.Freq.Control*, 54, 1560-1570.

Laube, S., Henning, M., Brandt, H., Kallweit, E. and Glodek, P. (2000). Die Fleischbeschaffenheit von Schweinekreuzungen mit besonderen Qualitätseigenschaften im Vergleich zum heutigen Standard- und Markenschweineangebot. *Arch.Tierz., Dummersdorf*, 43, 463-476.

Laugier, P. (2008). Instrumentation for in vivo ultrasonic characterization of bone strength. *IEEE Trans Ultrason Ferroelectr Freq Control.*, 55, 1179-1196.

Leseigneur-Meynier, A. and Gandemer, G. (1991). Lipid composition of pork muscle in relation to the metabolic type of the fibres. *Meat Science*, 29, 229-241.

LFGB (2005). Bestimmung des Gesamtfettgehaltes in Fleisch und Fleischerzeugnissen. *Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB: L 06.00 6*.

Link, G. (2007). Beziehungen zwischen objektiv und subjektiv ermittelten Fleischqualitätsparametern unter besonderer Berücksichtigung der Muskelstrukturmerkmale im *Musculus longissimus* von Schweinen. In : Ph.D. Thesis, Department of animal science, Georg-August-University Göttingen.

Lizzi, F. L., Feleppa, E. J., Alam, S. K. and Deng, C. X. (2003). Ultrasonic spectrum analysis for tissue evaluation. *Pattern Recognition Letters*, 24, 637-658.

Miles, C. A. and Fursey, G. A. (1977). Measurement of the fat content of meat using ultrasonic waves. *Fd.Chem.*, 2, 107-118.

Miles, C. A. and Fursey, G. A. J. (1974). A note on the velocity of ultrasound in living tissue. *Anim.Prod.*, 18:93.

Miller, L. R., Garwood, V. A. and Judge, M. D. (1975). Factors affecting porcine muscle fiber type, diameter and number. *Journal of Animal Science*, 41, 66-77.

Mörlein, D., Link, G., Werner, C. and Wicke, M. (2007). Suitability of three commercially produced pig breeds in Germany for a meat quality program with emphasis on drip loss and eating quality. *Meat Science*, 77, 504-511.

Mörlein, D., Rosner, F., Brand, S., Jenderka, K. V. and Wicke, M. (2005). Non-destructive estimation of the intramuscular fat content of the *longissimus* muscle of pigs by means of spectral analysis of ultrasound echo signals. *Meat Science*, 69, 187-199.

Nair, A., Kuban, B. D., Obuchowski, N. and Vince, D. G. (2001). Assessing spectral algorithms to predict atherosclerotic plaque composition with normalized and raw intravascular ultrasound data. *Ultras.in Med & Biol*, 27, 1319-1331.

Nassiri, D. K., Nicholas, D. and Hill, C. R. (1979). Attenuation of ultrasound in skeletal muscle. *Ultrasonics*, 17, 230-232.

Newcom, D. W., Baas, T. J. and Lampe, J. F. (2002). Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *Journal of Animal Science*, 80, 3046-3052.

Ngapo, T. M., Babare, I. H., Reynolds, J. and Mawson, R. F. (1999). Freezing and thawing rate effects on drip loss from samples of pork. *Meat Science*, 53, 149-158.

Oelze, M. L., Zachary, J. F. and O'Brien, W. D. (2002). Characterization of tissue microstructure using ultrasonic backscatter: Theory and technique for optimization using a Gaussian form factor. *J.Acoust.Soc.Am.*, 112, 1202-1211.

Offer, G. and Trinick, J. (1983). On the Mechanism of Water Holding in Meat: The Swelling and Shrinking of Myofibrils. *Meat Science*, 8, 245-281.

Park, B., Whittaker, A. D., Miller, R. K. and Hale, D. S. (1994). Predicting Intramuscular Fat in Beef Longissimus Muscle from Speed of Sound. *Journal of Animal Science*, 72, 109-116.

Raum, K. (2008). Microelastic imaging of bone. *IEEE Trans Ultrason Ferroelectr Freq Control.*, 55, 1417-1431.

Rosenvold, K. and Andersen, H. J. (2003). The significance of pre-slaughter stress and diet on colour and colour stability of pork. *Meat Science*, 63, 199-209.

Ryu, Y. C. and Kim, B. C. (2005). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig *longissimus dorsi* muscle. *Meat Science*, 71, 351-357.

Scheipers, U., Ermert, H., Sommerfeld, H. J., Garcia-Schürmann, M., Senge, T. and Philippou, S. (2003). Ultrasonic multifeature tissue characterization for prostata diagnostics. *Ultras.in Med & Biol*, 29, 1137-1149.

Shore, D., Woods, M. O. and Miles, C. A. (1986). Attenuation of ultrasound in post rigor bovine skeletal muscle. *Ultrasonics*, 24, 81-87.

Smith, N. B. (1996). Effect of myofibril length and tissue constituents on acoustic propagation properties of muscle. *Ph.D.Thesis, University of Illinois.*

Strelitzki, R., Clarke, A. J. and Evans, J. A. (1996). The measurement of the velocity of ultrasound in fixed trabecular bone using broadband pulses and single-frequency tone bursts. *Phys.Med.Biol.*, 41, 743.

Suster, D., et al. (2003). Accuracy of dual energy X-ray absorptiometry (DXA), weight and P2 back fat to predict whole body and carcass composition in pigs within and across experiments. *Livestock production science*, 84, 231-242.

Topp, K. A. and O'Brien, W. D. Jr. (2000). Anisotropy of ultrasonic propagation and scattering properties in fresh rat skeletal muscle in vitro. *J.Acoust.Soc.Am.*, 107, 1027-1033.

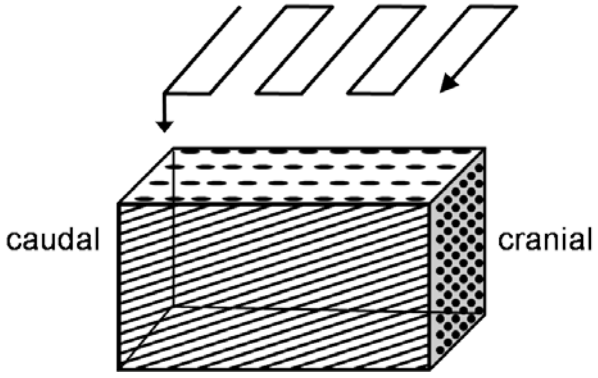


Figure 1: Sound propagation direction (arrows) relative to the muscle fibre orientation.

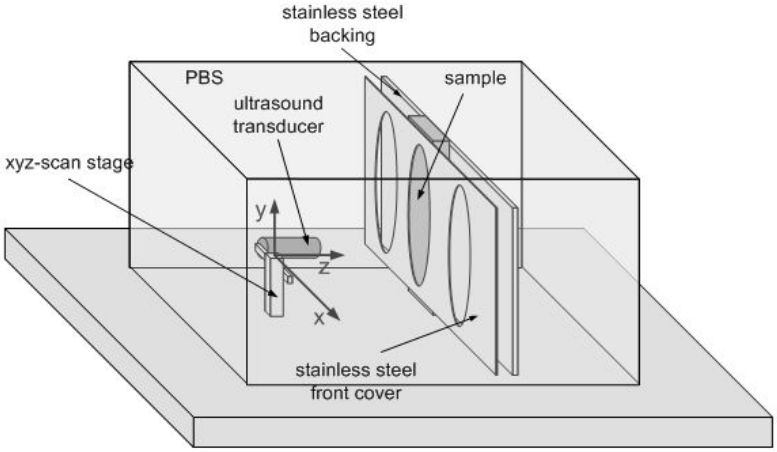
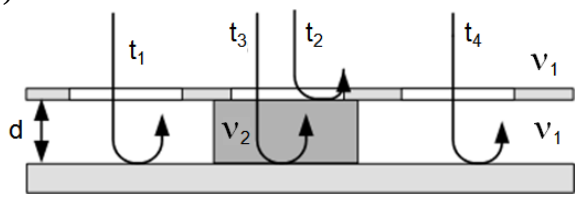


Figure 2: Scan set-up. Samples are placed in the central chamber. The transducer is moved in x and y direction over a scan field that covers the sample chamber and the two adjacent reference chambers. 150 A-scans are acquired within the sample chamber.

a)



b)

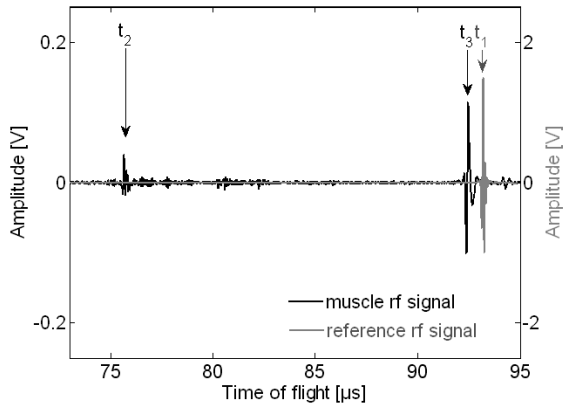


Figure 3: Recorded echo positions used for calculation of the sound velocity (a).
t_1, t_4: travel times in PBS; t_2: pulse position of the front echo; t_3: travel time within muscle sample; ν_1: sound velocity in PBS; ν_2: sound velocity in tissue sample. Exemplary pulse-echo signal for the reference and muscle tissue propagation paths are shown in (b).

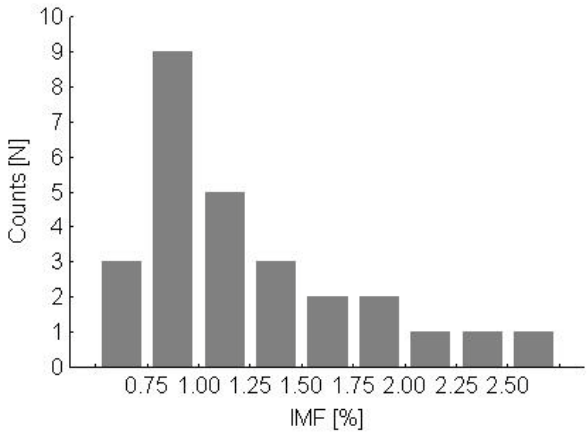


Figure 4: Distribution of the IMF values in the evaluated porcine *longissimus* muscle (n = 27).

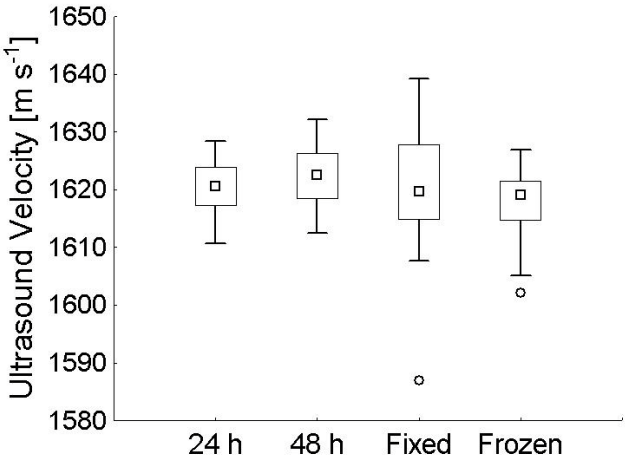


Figure 5: Ultrasound velocity of porcine longissimus muscle samples with respect to sample preparation (n = 27; F = 2.62). 24, 48: native samples, measured 24 and 48 h. p.m.; Fixed: fixed in formaldehyde 24 h. p.m. and washed in PBS 12 h before measurement; Frozen: samples frozen 24 h. p.m. and thawed before measurement.

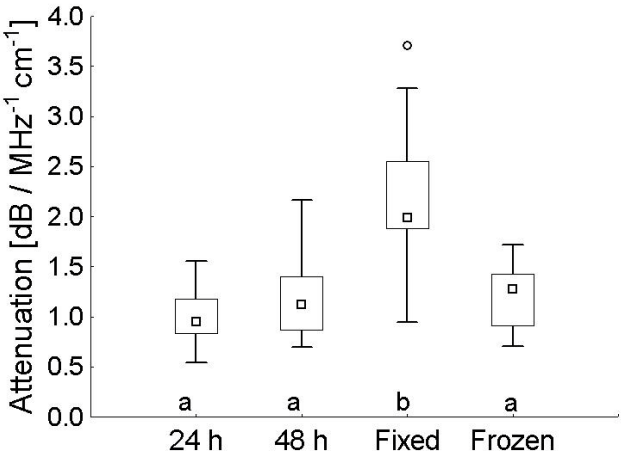


Figure 6: Variation of acoustic attenuation of muscle samples with respect to sample preparation (n=27).
a,b: Different letters indicate significant differences between treatments (F = 53.56).
24, 48: native samples, measured 24 and 48 h. p.m.; Fixed: fixed in formaldehyde 24 h. p.m. and washed in PBS 12 hrs before measurement; Frozen: samples frozen 24 h. p.m. and thawed before measurement.

Table 1: Descriptive statistics (mean and standard deviation, coefficient of variation (CV) in percent) of carcass and meat quality characteristics, histological and ultrasound parameters.

	Mean	CV [%]	Minimum	Maximum
Hot carcass weight [kg]	94 ± 6	6.8	80	108
Lean meat [%]	55 ± 5	8.4	46	61
Backfat width [mm]	18 ± 5	26.8	11	26
Muscle width [mm]	61 ± 6	10.6	43	73
pH 45	6.4 ± 0.2	2.8	6.1	6.7
EC 45 [mS/cm]	3.6 ± 0.4	9.9	3.1	4.8
L*	47.1 ± 2.8	6.0	41.3	53.4
a*	6.3 ± 1.4	21.9	3.8	9.1
b*	0.4 ± 0.7	180.6	-1.0	1.4
Intramuscular fat [%]	1.3 ± 0.6	45.7	0.6	3.2
Drip loss [%]	5.7 ± 2.9	51.9	1.3	13.4
Thawing loss [%]	6.6 ± 1.9	28.6	3.7	10.7
Protein [%]	24.0 ± 0.8	3.5	21.8	25.4
Dry matter [%]	25.5 ± 0.7	2.7	24.4	27.0
Bundle Ø [µm]	806 ± 115	14.3	532	1103
Fibre Ø [µm]	81 ± 7	9.4	68	95
FTG Ø [µm]	85 ± 8	9.9	68	102
FTO Ø [µm]	65 ± 10	14.6	45	96
STO Ø [µm]	70 ± 7	10.3	55	81
FTG [%]	78 ± 4	4.9	71	84
FTO [%]	12 ± 3	28.8	6	19
STO [%]	10 ± 3	29.2	6	18
Sound speed [ms^{-1}]				
native, 24 h p.m.	1620 ± 4.6	0.29	1610	1628
native, 48 h p.m.	1622 ± 5.2	0.32	1612	1632
frozen-thawed	1617 ± 6.2	0.39	1602	1626
formalin fixed	1619 ± 10.7	0.66	1587	1639
Attenuation [dB MHz^{-1} cm^{-1}]				
native, 24 h p.m.	1.02 ± 0.26	25	0.54	1.55
native, 48 h p.m.	1.16 ± 0.34	29	0.70	2.17
frozen-thawed	1.19 ± 0.29	25	0.70	1.72
formalin-fixed	2.23 ± 0.59	27	0.95	3.71

Table 2: Pearson product moment correlations between meat quality traits, ultrasound velocity and attenuation of porcine *longissimus* muscle with respect to measurement condition (N=27).

	IMF	pH	Drip loss	Protein	Dry matter
ultrasound velocity					
native, 24 h p.m.	-0.50 **	-0.04	0.17	0.41 *	-0.06
native, 48 h p.m.	0.01	0.02	-0.05	0.18	0.34
frozen-thawed	-0.16	0.15	-0.03	0.52 **	0.29
formalin-fixed	-0.35	-0.11	0.02	0.32	0.09
attenuation					
native, 24 h p.m.	0.66 ***	0.33	-0.43 *	-0.15	0.35
native, 48 h p.m.	0.67 ***	0.29	-0.18	-0.33	0.23
frozen-thawed	0.40 *	0.40 *	-0.27	-0.13	0.16
formalin-fixed	0.58 **	0.41 *	-0.57 **	-0.34	0.23

*p<0.05; **p<0.01; ***p<0.001

Table 3: Variations of acoustic properties (mean values and standard deviation) in native samples with respect to the IMF class. In addition to the distinct ageing groups the results of the combined ageing groups are provided.

	F-value	Low (< 1.0 %)	Mid (1.0 – 1.58 %)	High (> 1.58 %)
N		12	9	6
Velocity (24 h) [ms^{-1}]	2.44	1622 ± 4 [a]	1620 ± 4 [ab]	1617 ± 6 [b]
Velocity (48 h) [ms^{-1}]	0.33	1622 ± 5	1623 ± 5	1623 ± 6
Velocity (combined) [ms^{-1}]	0.62	1622 ± 4	1622 ± 5	1620 ± 7
Attenuation (24 h) [dB MHz^{-1} cm^{-1}]	8.04	0.88 ± 0.19 [a]	1.04 ± 0.24 [b]	1.29 ± 0.19 [c]
Attenuation (48 h) [dB MHz^{-1} cm^{-1}]	4.94	1.05 ± 0.26 [a]	1.08 ± 0.24 [a]	1.50 ± 0.44 [b]
Attenuation (combined) [dB MHz^{-1} cm^{-1}]	11.73	0.96 ± 0.24 [a]	1.07 ± 0.23 [a]	1.40 ± 0.34 [b]

[abc]: Different letters indicate significant differences within a line.

Paper II:

Koch, T.; Lakshmanan, S.; Brand, S.; Wicke, M.; Raum, K.; Mörlein, D.

Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Skin and backfat

Meat Science

Accepted: 3. December 2010

DOI: 10.1016/j.meatsci.2010.12.004

Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: II. Skin and backfat

Koch, Tim *[a]; Lakshmanan, Sannachi *[b]; Brand, Sebastian [c]; Wicke, Michael [a]; Raum, Kay [b, d] and Mörlein, Daniel [a+]

[a]Department of Animal Sciences, University of Goettingen, Germany

[b]Julius Wolff Institute and Berlin-Brandenburg School for Regenerative Therapies, Charité-Universitätsmedizin, Berlin, Germany

[c]Fraunhofer Institute of Material Mechanics, Halle, Germany

[d]Department of Orthopedics, Martin Luther University of Halle-Wittenberg, Halle, Germany

* Both authors equally contributed to the study.

[+] corresponding author

Ultrasound is regarded as a promising method to determine the intramuscular fat content of pork loin. At intact carcasses, the signal passes the backfat whose ultrasound parameters (sound velocity, attenuation) have not been fully investigated. This study intended to collect a dataset of ultrasound parameters for individual backfat layers and to elucidate relationships with structural and compositional characteristics. In-vitro measurements at 10 MHz were conducted on backfat samples of pork carcasses representative for German populations. The average sound velocity ranged from 1436 ± 9 to 1470 ± 37 ms^{-1} for the fat layers, and 1682 ± 23 ms^{-1} for skin. Velocity of the compound backfat decreased with overall thickness. Attenuation was not affected by thickness ranging between 1.6 ± 0.7 and 2.7 ± 1.5 dB MHz^{-1} cm^{-1} for all layers. Sound velocity was negatively correlated with fat content and dry matter. The obtained results are anticipated to improve signal correction prior to spectral analysis of ultrasound measurements at intact carcasses.

Keywords: Ultrasound, attenuation, sound velocity, longissimus, pig, backfat, skin, fatty acid composition

1. Introduction

The intramuscular fat content (IMF) is widely regarded as an important parameter influencing the sensory characteristics of pork loin (Fernandez, Monin, Talmant, Mourot & Lebret, 1999). Therefore, many investigations targeted at non-invasive estimation of the fat content inside the muscle at intact pig carcasses. Several of these methods, including the use of ultrasound and spectral analysis, have been described in more detail in part I of this study (Koch, Brand, Lakshmanan, Raum, Wicke & Mörlein, 2010). Although a previous study reported an average prediction error of 0.36 % IMF using spectral analysis of unprocessed backscatter signals (Mörlein, Rosner, Brand, Jenderka & Wicke, 2005), this is not yet satisfying for use in an industrial slaughterhouse.

Our current work is focused on the improvement of the precision of ultrasound based estimations of IMF. Towards this goal, precise estimations and corrections for variations of sound velocity and attenuation for all involved tissues on the sound propagation path between the ultrasound transducer and the evaluated muscle tissue are necessary.

Ultrasound velocity and/or attenuation of porcine fat and skin have been reported earlier (Chivers and Parry, 1978; Greenleaf, 1986). However, none of the investigations provided a dataset applicable for in-vivo conditions (45 min p.m.), e.g. with respect to temperature that is known to largely affect the results (Ninoles, Clemente, Ventanas & Benedito, 2007) and that takes the compositional variation into account.

Backfat structure and composition that also impacts on its mechanical and chemical quality (e.g. solidity and oxidative stability), were reported to be remarkably affected by sex, breed type and diet (Brooks, 1971; Wood, Nute, Richardson, Whittington, Southwood, Plastow, Mansbridge, da Costa & Chang, 2004; Daza, Ruiz-Carrascal, Olivares, Menoyo & López-Bote, 2007; Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes & Whittington, 2008). In addition to inter-individual variations, the fatty acid profile, i.e. the amount of saturated and unsaturated fatty acids, also varies within animals between the outer (subcutaneous), intermediate, and inner (muscle side) porcine backfat layers (Daza et al., 2007). Moisture and fat content have been stated to differ between individual layers and with respect to backfat thickness (Moody and Zobrisky, 1966).

In this study, acoustic attenuation and sound velocity reference data of porcine backfat including skin were collected *in-vitro* in a set of pig carcasses resembling a variation of carcass properties typically found in German slaughter facilities. Variations of the acoustic properties were analyzed with respect to structural and compositional variations. Temperature

and direction of the incident sound waves were adjusted to close-to in-vivo conditions. To avoid any influence of directional dependency (anisotropy) stated for different types of tissue (e.g. muscle (Smith, 1996)) the direction of the sound propagation was adjusted to be comparable to measurements at hanging carcasses.

2. Materials and methods

2.1. Animals, meat quality parameters and chemical determinations

A representative variation of tissue properties was obtained by selection of pig carcasses from a commercial abattoir (n = 27, left half each) according to 3 levels (low, mid, high) of carcass weight and lean meat percentage (LMP), with 3 animals for each group. All preparation steps and analyses of muscle tissue prior to the excision of the backfat layer are described in our companion paper (Koch et al., 2010), hereinafter referred to as part I. Approximately 24 h p.m. after chilling of the carcasses over night, one chop was excised from the *m. longissimus* at the $2^{nd}/3^{rd}$ last rib for subsequent laboratory analyses. All samples were kept in the refrigerator at 4 °C until ultrasound measurements that were performed within 72 to 96 h p.m.. Prior to the acoustic examination 2 adjacent samples were cut about 7 cm from the split line and prepared as follows: both samples were cut to a side length of about 1.5 cm and the remaining muscle tissue was removed. The first sample was measured as an intact backfat block including skin (compound sample). The second sample was dissected, and fat layers and skin were measured individually after removing the connective tissue between the layers. Dry matter determination was performed at small sample pieces (~ 5 g) that were dried in sea sand at a temperature of 80 °C until equilibrium weight was reached. All chemical analyses were related to fresh matter weight.

The fatty acids of the individual fat layers of 20 backfat samples were analysed via gas chromatography (GC) Varian 3400 (Varian GmbH; Darmstadt, Germany) using the autosampler Varian 8200 CX (Varian GmbH; Darmstadt, Germany) and a SP-2340 fused-silica capillary column (Polymicro Technologies; Phoenix AZ; USA). Prior to GC the samples were methylated with bortrifluoride-methyl-alcohol at 110 °C for 3 h (Scheeder, Gläser, Eichenberger & Wenk, 2000). Heptadecanoic acid (C:17) was used as internal standard. The results were analysed using the software GC-star V.A2 (Varian GmbH; Darmstadt, Germany). Averaged results of 2 replicate analyses were used for further work. Individual fatty acids are given as percent of total detected fatty acids, and summarized as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

2.2. Ultrasound data acquisition

Ultrasonic data acquisition was performed using a custom made scanning acoustic microscope (SAM) as described in part I of this study. Prior to measurement the samples were allowed to equilibrate in phosphate buffered saline (PBS) at 38 °C as an isotonic medium. The PBS solution was prepared using 9.55 g/L Dulbeccos powder (AppliChem; Darmstadt, Germany), that consists mainly of NaCl (80%) and Na_2HPO_4 (15%) resulting in an overall concentration of slightly less than 0.15 M.

Each sample was placed in a custom made multi-chamber-holder (see part I). This chamber holder allowed the acquisition of pulse echoes from a plane steel reflector with and without sample, as well as tight attachment of the sample without compression. The scans were performed with a step width of 0.4 by 1 mm, resulting in approximately 150 radio frequency (rf) lines per chamber. For acoustic coupling, the sample and the transducer were submerged in degassed PBS at 38.0° ± 0.1 °C (sound velocity 1539 ms^{-1}). The estimation of sound velocity and acoustic attenuation from the ultrasonic echo signals is described in part I of this study.

Prior to signal analysis, a region of interest (ROI) in the central part of the chamber was manually selected resulting in approximately 100 rf-lines. For each rf-signal the local sound velocity was estimated. Individual rf pulse-echo signals with a discrepancy of more than 1.5 times of the standard deviation of the mean velocity estimate were excluded from further analyses. These outliers were mainly caused by a partial detachment of the sample from the backplate. Finally, between 60 and 80 rf-signals per sample were used for estimating speed of sound and frequency dependent attenuation. Each data set was analyzed by two independent trained users to estimate the effects in terms of software handling. The mean differences between both users were less than 0.1 % for sound velocity and less than 5 % for attenuation.

2.3. Statistical analysis

The statistical analysis was performed with SAS 9.1 (SAS Institute, Cary, USA), STATISTICA 7.1 (StatSoft, Tulsa, USA) and The Unscrambler 9.2 (CAMO ASA, Oslo, Norway). To evaluate differences between individual layers, first all samples were checked for normal distribution performing a Shapiro-Wilks test in SAS. Thereafter, analysis of variance (ANOVA) followed by post-hoc multicomparison Tukey-tests were applied. Linear

regression and Pearson product-moment correlation coefficients were used to study the associations of acoustic parameters with structural and compositional parameters.

The best combination of structural and compositional properties for predicting the variations of the acoustic properties was evaluated using multifactorial linear regression (MLR) analysis using STATISTICA and UNSCRAMBLER. In all cases full-cross validation was used to verify the results. All statistical results were considered significant for p values smaller than .05.

3. Results

3.1. Carcass characteristics and backfat composition

Descriptive statistics of carcass traits are summarized in part I of this study. Briefly, the average carcass weight and lean meat percentage (LMP) were 94 ± 6 kg and 55 ± 5 %, respectively. Muscle and backfat widths ranged from 42.7 to 73.0 mm and 11.2 to 25.9 mm. The IMF content was widely spread from 0.63 to 3.16 % with a mean value of 1.29 %.

Table 1 summarises thickness and chemical composition of all 3 fat layers. Significantly higher thickness values were found for the subcutaneous and intermediate fat layer compared to the inner layer. The skin thickness was 2.64 ± 0.36 mm. The thickness of the compound sample was mainly influenced by the thickness of the subcutaneous and intermediate layers. Predicting the compound thickness via MLR resulted in an R^2 value of .89 and a RMSE of 1.36 mm using only the thickness of those two layers (not shown). In contrast to this, variations of skin and inner layer thicknesses were not associated with variations of the compound thickness. Dry matter percentage was lower in the inner layer than in the other layers. No differences were observed for the total amounts of fatty acids. The subcutaneous fat layer exhibited a lower relative amount of SFA and higher amounts of MUFA and PUFA than the other layers.

Correlations between carcass traits and the chemical composition of all 3 fat layers are summarized in Table 2. An increase in backfat width was accompanied by a significant increase of fatty acids (up to r = .85) and dry matter (up to r = .72) in all fat layers with highest correlations for the intermediate fat layer. Negative relationships were observed between the lean meat percentage and the total amount of fatty acids (up to r = -.73) and dry matter (up to r = -.66) for individual layers. The IMF was moderately correlated with the total amount of fatty acids of the inner backfat layer (r = .59). A negative relation between the IMF and the amount of PUFA was observed in the subcutaneous fat layer (r = -.49).

3.2. Ultrasound parameters

Ultrasound velocity and attenuation values (median, upper and lower quartile) for the compound sample (all three layers and skin), skin and the 3 individual fat layers are given in Figs. 1 and 2. ANOVA revealed significant differences of the sound velocity between all evaluated groups (F = 612). The velocity in skin (1682 ± 23 ms^{-1}) was considerably higher than in the individual fat layers. In fat, the velocity increased from the subcutaneous to the inner fat layer. The velocity values of the compound samples were between the values observed for fat and skin.

The differences of attenuation between the groups were less pronounced compared to those of the sound velocity (F = 8.1). Slightly reduced values were observed in the subcutaneous and intermediate layers compared the inner fat layer, skin, and the compound samples. It should be noted that variations within the groups were also much higher than those found for the sound velocity.

3.3. Relationships of ultrasound parameters with carcass and backfat characteristics

Relationships between carcass traits and ultrasound parameters are presented in Table 3. A strong decrease in sound velocity of the compound sample was found with increasing backfat width (r = -.80). The analysis within the individual layers revealed that the negative association of sound velocity and layer thickness only occurred in the intermediate and inner fat layers. In the intermediate layer, the thickness was also inversely related to the acoustic attenuation.

Table 4 summarises the relationships between ultrasound parameters and chemical composition of the fat layers. The sound velocity decreased with increasing amount of fatty acids (up to r = -.53) and dry matter (up to r = -.65). These moderate effects within the individual layers were confirmed after pooling the data for all groups. Moreover, sound velocity exhibited moderate correlations with the amount of SFA (r = .29). Using MLR to predict sound velocity of the individual fat layers (Eqs. 1 and 2) revealed that DM was the most important compositional parameter for both, the subcutaneous (R^2 = .08; RMSE: 9.08 ms^{-1}) and the intermediate fat layer (R^2 = .30; RMSE: 18.38 ms^{-1}). Comparable results were found for the pooled data of all backfat layers (Eq. 3) with DM again being the only included compositional parameter (R^2 = .09; RMSE: 23.09 ms^{-1}). Correlations between DM, thickness (up to r = .77) and FA (up to r = .70) precluded the implementation of additional parameters into the MLR model of individual fat layers or the pooled data. No MLR model could be

given for the inner fat layer as no compositional parameter reached the required level of significance.

$$v_{subcutaneous} = 1613.0\ ms^{-1} - 2.0\ ms^{-1} \cdot DM\ , \quad (1)$$

$$v_{intermediate} = 1798.0\ ms^{-1} - 3.9\ ms^{-1} \cdot DM\ , \quad (2)$$

$$v_{all} = 1680.0\ ms^{-1} - 2.6\ ms^{-1} \cdot DM\ . \quad (3)$$

Attenuation was not affected by the evaluated chemical composition, except for the MUFA content in the subcutaneous layer (Table 4). In the pooled data, a moderate negative correlation with dry matter (r = -.37) content was observed. As observed for sound velocity neither the calculated total number of double bonds nor the average fatty acid chain lengths showed a significant influence on attenuation (data not shown).

The relative impacts of backfat thickness and chemical composition on the acoustic properties of the compound samples could only partially be confirmed be stepwise multiple linear regression analysis. The sound velocity could be predicted by a combination of the backfat width (BW) and the amount of PUFA in the subcutaneous fat layer (R^2 = .79; RMSE = 9.1 ms^{-1}):

$$v_{compound} = 1496 - 2.86 mm^{-1}\ ms^{-1} \cdot BW + 2.34\ ms^{-1} PUFA_{subcut.}\ , \quad (4)$$

The squared semi partial correlation coefficients provide a measure of the contribution of each parameter to the variance explained by the model. 34.4 % and 12.9 % of the variance of the observed compound velocity are explained by variations of BW and PUFA, respectively. Consequently, including only the backfat width into the model revealed a slightly weaker prediction model (R^2 = .68; RMSE = 11.6 ms^{-1}).

$$v_{compound} = 1555 - 3.94 mm^{-1}\ ms^{-1} \cdot BW\ . \quad (5)$$

No significant multivariate model was found for the attenuation values.

4. Discussion

4.1. Backfat structure and composition

Fatty acid distribution in monogastric animals is largely affected by breed type, diet or production system (Daza et al., 2007; Ninoles et al., 2007). Within the present study, distributions of the fatty acids and fatty acid groups confirm earlier results at German pig populations. In a previous investigation of more than 600 pigs of three commercial crossbreds (Link, 2007) we observed on average 19.1 % PUFA, 44.3 % MUFA, and 36.6 % SFA in

backfat (subcutane and intermediate layer). A highly comparable amount has been stated for the 4 major fatty acids (palmitic, stearic, oleic and linoleic acid) of pigs from an industrial slaughterhouse, analysing the two outer fat layers combined (Davanel, Riaublanc, Marchal & Gandemer, 1999). Thus, the selected carcasses are suggested to be a representative sample with respect to commercial German slaughter pigs in terms of fatty acid composition.

As for the variation between the individual layers, a higher amount of moisture and a corresponding lower amount of fatty acids in the inner layer was stated (Moody and Zobrisky, 1966). This is partly in accordance with our results, whereas the inner layer had the significantly lowest amount of dry matter. Contrarily, no significant differences in the total fat content could be found between the layers. Varying fatty acid composition between fat layers was observed earlier. Lower levels of SFA but higher levels of MUFA and PUFA were found for the subcutaneous layer compared to the intermediate and inner layer (Daza et al., 2007; Monziols, Bonneau, Davanel & Kouba, 2007). This could be confirmed by our investigation. The observed relative thickness differences of the individual fat layers are also in agreement with previous studies (Moody and Zobrisky, 1966; Fortin, 1986; Müller and Polten, 2004).

Close relationships between the total backfat depth and the composition of the fat layers (i.e. the total amount of fatty acids) have already been stated in literature (Moody and Zobrisky, 1966; Davanel et al., 1999; Dinh, Blanton Jr., Riley, Chase Jr., Coleman, Phillips, Brooks, Miller & Thompson, 2010). Positive correlations between fat thickness and the amount of dry matter (Moody and Zobrisky, 1966) can be confirmed by our investigation. However, the relationships between the backfat width and fatty acid composition were less clearly pronounced. Increasing SFA and decreasing PUFA with increasing fat depth were published for backfat (Wood, 1973; Villegas, Hedrick, Veum, McFate & Bailey, 1973; Lo Fiego, Santoro, Macchioni & De Leonibus, 2005). Contrarily, recent investigations stated no significant relationships between backfat width and fatty acid composition in porcine adipose tissue (Furman, Malovrh, Levart & Kovac, 2010). Our results can be considered to be in accordance with earlier results, as minor significant positive relationships between BW and SFA were found for the inner layer. Furthermore, significant positive correlations were found between BW and the weighted amounts of SFA of all 3 fat layers ($r = .51$), while the amount of PUFA showed negative relationships ($r = -.46$) (data not shown).

4.2. Ultrasound parameters

Even if a wide variety of porcine tissues was investigated with ultrasound through the last decades (Chivers and Parry, 1978; Greenleaf, 1986; Ninoles et al., 2007), none of the

investigations provided a dataset comparable to conditions used during this investigation. In earlier investigations one or more important factors (e.g. temperature, measurement position and frequency) were either different from those of our study or not provided at all, and thus rendering a direct comparison impossible.

Within the present study the mean and standard deviation of sound velocity estimates in fat was 1450 ms^{-1} ± 27 ms^{-1} considering all individual fat layers. Earlier, a velocity of about 1530 ms^{-1} for freeze-thawed porcine backfat samples measured without skin at 20°C was stated by Ninoles et al. (2007). The difference can be explained by a negative temperature coefficient of ultrasonic velocity of solid and liquid fats (Benedito, Carcel, Rossello & Mulet, 2001; Ninoles, Sanjuan, Ventanas & Benedito, 2008) resulting in a non-linear decrease of sound velocity of about 6 to 7 ms^{-1} $°C^{-1}$ at 20°C (Ninoles et al., 2007). Furthermore, the reduction of the solid-to-liquid ratio due to the melting of fatty acids with increasing temperature also reduces sound velocity (McClements and Povey, 1992; Ninoles et al., 2007). Taking this into account, a velocity of about 1450 ms^{-1} at 38 °C is feasible.

The highest sound velocity obtained for the inner fat layer is probably caused by the higher water content compared to the other layers. The sound velocity in water increases with temperature, while the sound velocity in fat decreases (Del Grosso and Mader, 1972; McClements and Povey, 1992). At temperatures above approximately 24 °C the sound velocity of fat is lower compared to water (Miles and Fursey, 1977). Thus, sound velocity of fat tissue samples with higher water content (inner fat layer) is suggested to be higher when measured at temperatures above 24 °C. This is supported by the negative relationship between dry matter and sound velocity found for the fat layers in this investigation. Compared to the total amount of dry matter and fatty acids, the corresponding fatty acid composition seems to be less significant in terms of sound velocity. Ninoles (2007) however stated influences of both the amount of MUFA and PUFA on sound velocity. Those differences to our results may be caused by different temperatures used in both studies. Several fatty acids, including oleic acid accounting for about 40 % in our investigation, have been stated to melt at about 5 to 15 °C (Cedeno, Prieto, Espina & García, 2001). Therefore, an influence on sound velocity due to a change of the solid-to-liquid ratio observed in the temperature range between 0 to 20 °C may be negligible at 38 °C, when most of the fatty acids exist in liquid form. Furthermore, the differences in fatty acid composition between the samples were comparably low.

The obtained sound velocity values of skin (1682 ± 23.36 ms^{-1}) were slightly lower than previous findings. Values of 1710 ms^{-1} ± 60 ms^{-1} have been measured in porcine skin 3 days p.m. (Cantrell, Goans & Roswell, 1978). However, these measurements were performed

at lower temperatures (25 °C). Only 2 animals were investigated while the exact anatomical origin of the samples was not reported. Differences in the amount and cross-linking of the skin collagen between different breeds and body regions have been reported (Meyer, Neurand & Radke, 1982) and thus could explain the differences to our data.

To our knowledge, no investigation targeting the attenuation of the individual fat layers has been published. Gammell (1979) stated attenuation values of about 2.0 to 2.5 dB MHz^{-1} cm^{-1} for compound fresh porcine backfat samples obtained from one single animal at 37 °C. This is in the range of the present results, where attenuation values from 1.59 to 2.72 dB MHz^{-1} cm^{-1} were obtained.

The higher attenuation value measured for the inner fat layer is suggested to be a confounded effect of both the lower dry matter and the higher PUFA content compared to the other layers. Ultrasonic properties of fats and oils were shown to be affected by structural parameters such as fatty acid chain length, degree of saturation, melting point, and correspondingly the solid fat content (Hustad, Richardson, Winder & Dean, 1970; McClements and Povey, 1992; Maleky, Campos & Marangoni, 2007). Varying amounts of SFA and PUFA, and correspondingly the amount of double bonds, are anticipated to affect the attenuation of edible fats and oils.

For porcine skin, attenuation values of about 0.7 dB MHz^{-1} cm^{-1} have been estimated in a frequency range from 7 to 13 MHz at 25 °C (Cantrell et al., 1978). However, only 2 animals were used in that investigation. Our study has covered a broader range of tissue properties, whereas the samples with the smallest attenuation values (0.8 dB MHz^{-1} cm^{-1}) were comparable to those reported by Cantrell et al. (1978). Furthermore, relationships between the attenuation and the amount of collagen (up to r = .56) and water (up to r = -.73) have been stated for dog skin (Olerud, O'Brien, Riederer-Henderson, Steiger, Forster, Daly, Ketterer & Odland, 1987). Large differences between fibre arrangement and the amount of collagen were found with respect to anatomic origin and pig breed type (Meyer et al., 1982). Therefore, differences in body region, breed and age have to be considered for a direct comparison.

4.3. Correlations of ultrasound parameters with carcass and backfat characteristics

The relative proportion of the skin thickness versus compound thickness (12. 6 – 35.5 %) is the most important factor determining the velocity of the compound backfat (r = .89). This is explained by the significantly higher sound velocity of skin tissue compared to those of the backfat layers (1682 ms^{-1} vs. 1450 ms^{-1}). The thickness of the skin revealed only slight

differences between samples while there was no relationship to the thickness or velocity of the compound sample. Therefore, the total backfat thickness can be assumed to be independent on variations of the thickness of the skin, while the relative proportion of skin compared to the total backfat thickness increases with decreasing backfat depth. Contrarily, the individual fat layers show a comparably wide range of thickness variation (Fig. 3), mainly affecting the thickness of the backfat compound. As shown by MLR (Eqs. 4 and 5) the compound thickness and with it the thickness of the individual fat layers is mainly accounting for the sound velocity of the compound sample. Out of the 3 fat layers the thickness of the intermediate one seems to be most important followed by the subcutaneous one. MLR revealed an R^2 value of .89 for predicting the compound thickness using only the thickness values of the subcutaneous and the intermediate layers. On the other hand, neither the thickness of the inner layer nor that of skin was associated with variations of the compound thickness. This can be explained by the development of the individual layers. The subcutaneous layer is developed first followed by the intermediate layer, while the inner layer is developed rather late and may therefore not be fully developed. Furthermore, the intermediate layer increases to a higher extend compared to the subcutaneous one and does therefore show higher differences between the animals (Moody and Zobrisky, 1966), which was confirmed by the higher standard deviation in our study.

In addition to the backfat thickness, the amount of PUFA in the subcutaneous layer was shown to significantly influence the sound velocity of the compound sample. This is in accordance to earlier investigations, were the fatty acid distribution has been suggested to influence sound propagation properties in fats and oils (McClements and Povey, 1992; Ninoles et al., 2007). Ninoles (2007) mentioned an increased sound velocity for backfat samples with higher amounts of MUFA and PUFA, which is accordance with our results. In terms of implementation of the amount of PUFA into the MLR model, both the PUFA of the subcutaneous and the intermediate fat layers reached the required level of significance. However, due to auto-correlations of the PUFA content in both layers ($r = .93$), only one layer was implemented in the model.

Even when PUFA as a compositional parameter is implemented in the model, the proportion of skin compared to total backfat thickness is the by far most important variable. Considering Eq. 4, the sound velocity of the compound sample can be expected to differ by less than 25 ms^{-1} between samples with low and high amounts of PUFA (i.e. from 12.6 % to 25.4 %). In contrast to alterations due to the fatty acid composition (e.g. PUFA), the observed differences in the compound thicknesses would cause variations of the sound velocity of more

than 45 ms^{-1}. As additional factors, the amounts of DM and FA have been shown to significantly increase the sound velocity within the individual fat layers. Both parameters however were not implemented in the MLR model. This again is explained by high correlations of both parameters with the backfat width (up to r = .85). While both parameters can be considered to influence the compound sound velocity, this is already covered by the effect of the increasing backfat thickness.

As shown in Fig. 4, the sound velocity can be predicted with reasonable accuracy (R^2 = .66; RMSE = 12 ms^{-1}) using only the compound backfat thickness. This is an important finding as the backfat thickness is being determined at slaughter facilities and can hence be implemented easily into ultrasound correction algorithms.

Furthermore, these findings are of economical interest, as the commonly used assumption of constant sound velocity for the skin-fat tissue compound will necessarily lead to an uncertainty in the backfat thickness estimation. Therefore, the incorporation of a thickness dependent sound velocity in ultrasound based grading systems is anticipated to significantly improve the estimation of backfat and muscle thickness and the subsequent lean meat yield calculation. Ideally, such systems need to detect not only the fat-muscle boundary, but also the skin-fat boundary. By this approach the lack of accuracy of ultrasound equipment for backfat thickness estimation, particularly with very lean carcasses (Müller and Polten, 2004) can be overcome.

The decreasing sound velocity with increasing thickness of the individual fat layers is mainly a consequence of the increasing amount of fatty acids and dry matter with increasing thickness. Both, the total amount of fatty acids and dry matter have been shown to have negative relationships to the sound velocity (up to r = -.65). Higher correlations of sound velocity with fatty acids and dry matter in the intermediate and inner layers are presumably caused by a higher variation of both variables in these layers (Moody and Zobrisky, 1966; Fortin, 1986). Earlier investigations also stated an influence of fatty acid composition on sound propagation properties in fats and oils (McClements and Povey, 1992; Ninoles et al., 2007). No comparable findings can be mentioned for our investigation besides a minor positive correlation between SFA and the sound velocity of the pooled fat layers. As mentioned before, the lack of relationships can be explained by procedural differences, mainly the temperature. Ninoles (2007) performed his measurements at a range from 0 to 20 °C compared to our investigations at about 38 °C, which most likely has affected the solid-to-liquid ratio of the fatty acids. Ninoles (2007) reported a decrease of sound velocity from 0 °C

to 20 °C due to the melting of the fatty acids as referred to by differential scanning calorimetry (DSC). The melting point of individual fatty acids is defined by the amount of carbon atoms and double bonds. The higher the number of carbon atoms and double bonds in fat, the lower the melting point and therefore also sound velocity (Ninoles et al., 2007). Lower solid-to-liquid ratios in oils and fats resulted in lower sound velocity values (McClements and Povey, 1988; McClements and Povey, 1992). This could not be confirmed by our study as we did not observe significant relationships between the number of double bonds or carbon atoms and sound velocity (results not shown). Besides the influence of temperature, this is probably due to the higher variation of water content between layers compared to the variation of fatty acid types. However, we did not use DSC to determine the solid fat content. Accordingly, only very few differences between the sound velocities of vegetable oils differing in fatty acid composition measured at about 20 °C were found (Javanaud and Rahalkar, 1988). Therefore, the small differences in the amount of double bonds and carbon atoms of backfat samples in our study may be negligible compared to the total amount of fatty acids and dry matter in terms of ultrasound velocity.

In contrast to sound velocity, no influence of the relative proportion of the skin could be stated for attenuation of compound backfat samples. The differences in attenuation between the skin (2.71 dB MHz^{-1} cm^{-1}) and the fat layers (1.62 to 2.72 dB MHz^{-1} cm^{-1}) are rather small and therefore have a much lower effect on the attenuation of the compound sample. Furthermore, no significant influence for any of the compositional parameters could be found. This lack of relationships may partially be explained by influences due to the used method. Firstly, dividing the layers may have slightly altered structure and state of compression, which may have an influence on the measurements. Furthermore, frequency dependent attenuation of the compound samples is highly affected by tissue boundaries. When the compound backfat sample is analysed, the sound wave has to traverse not only skin and the individual backfat layers, but also their interfaces that mainly consist of connective tissue. This effect is excluded when individual layers are analysed. An analysis of the amount of connective tissue has not been conducted, as the variance can be expected to be rather small compared to that of the other evaluated parameters (Wood, Enser, Whittington, Moncrieff & Kempster, 1989). However, the sound transmission losses at the individual interfaces may be highly dependent on subtle variations of composition, structure and thickness of the connective tissue interfaces. As attenuation of both, compound sample and individual fat layers can be considered to be mostly unaffected by the investigated structural

and compositional parameters of the fat tissue, the estimation of the backfat compound attenuation value, as proposed for sound velocity, could not be achieved. For this, the transmission losses at the individual tissue interfaces would need to be quantified. This however, seems to be impossible with current ultrasound devices used at slaughter facilities, as these layers are located very close to the transducer surface and within the near field of the common sound geometries used for this purpose.

5. Conclusions and implications

For the first time, a comprehensive survey of sound velocity and attenuation values for all 3 individual porcine backfat layers and skin was conducted with a representative sample of commercial pigs. The acoustic parameters were related to structural and compositional traits. Since the data are going to be used for ultrasound measurements at hot carcasses in commercial slaughterhouses the measurements were performed at 38 °C.

According to the results, the sound velocity of the compound sample is mainly influenced by the relative proportion of the skin compared to total backfat thickness. This is suggested to be mainly caused by the considerably higher sound velocity of skin compared to that of fat tissue. Thus, sound velocity is decreasing with increasing total backfat thickness. Additional parameters, e.g. the amount of fatty acids or dry matter significantly influence the sound velocity values of the fat layers. However, this influence is rather small compared to the proportion of the skin on total compound thickness. Furthermore, both parameters show medium to high correlations with the backfat thickness. Therefore, statistical models including the backfat thickness will inherently include the effects of both compositional parameters. Thus, in terms of ultrasound measurements a correction of sound velocity within the backfat compound seems to be feasible using only the backfat thickness. The remaining unexplained variance of the sound velocity (~ 32 %) can partially be described by the fatty acid distribution (e.g. PUFA) reaching about 11 % explained variance. No comparable statement could be given for the attenuation as i) the skin shows rather similar attenuation values compared to fat tissue and ii) the attenuation losses in the backfat compound appear to be predominantly affected by the connective tissue interfaces between the fat layers.

Compositional differences between the fat layers were found. The inner layer contains the lowest amount of dry matter while the subcutaneous layer contains more PUFA and correspondingly lower amounts of SFA. All 3 fat layers showed only slightly different sound velocity values while the attenuation was about doubled for the inner fat layer.

The sound velocity of the individual fat layers was negatively related to the amount of fatty acids and dry matter while no correlations to any of the fatty acid main groups could be found. In contrast, the amount of PUFA was the only compositional parameter included in the MLR model to predict the sound velocity of the compound sample.

The obtained information will be used to optimize an ultrasound based pig carcass grading device under development to estimate the intramuscular fat content of loin. The investigated relationships are anticipated to improve the signal correction procedures needed for ultrasound measurements at hot carcasses. The remarkably different sound velocities of skin and fat layers may be used for more precise backfat thickness estimations as long as the skin-fat-boundary is detectable with that specific ultrasound device.

Acknowledgements

This work was financially supported by the German Research Council (DFG; grants Mo 1746/1 and Ra 1380/3), PRO INNO (grant KF0380401LF6) and SFK Technology GmbH.

Reference List

Benedito, J., Carcel, J. A., Rossello, C. and Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 57, 365-370.

Brooks, C. C. (1971). Fatty acid composition of pork lipids as affected by basal diet, fat source and fat level. *J.Anim.Sci.*, 33, 1224-1231.

Cantrell, J. H. Jr., Goans, R. E. and Roswell, R. L. (1978). Acoustic impedance variations at burn-nonburn interfaces in porcine skin. *J.Acoust.Soc.Am.*, 64, 731-735.

Cedeno, F. O., Prieto, M. M., Espina, A. and García, J. R. (2001). Measurements of temperature and melting heat of some pure fatty acids and their binary and ternary mixtures by differential scanning calorimetry. *Thermochemica Acta*, 369, 39-50.

Chivers, R. C. and Parry, R. J. (1978). Ultrasonic velocity and attenuation in mammalian tissues. *J.Acoust.Soc.Am.*, 63, 940-953.

Davanel, A., Riaublanc, A., Marchal, P. and Gandemer, G. (1999). Quality of pig adipose tissue: relationship between solid fat content and lipid composition. *Meat Science*, 51, 73-79.

Daza, A., Ruiz-Carrascal, J., Olivares, A., Menoyo, D. and López-Bote, C. J. (2007). Fatty Acids Profile of the Subcutaneous Backfat Layers from Iberian Pigs Raised Under Free-range Conditions. *Food Sci Tech Int*, 13, 135-140.

Del Grosso, V. A. and Mader, C. W. (1972). Speed of Sound in Pure Water. *J.Acoust.Soc.Am.*, 52, 1442-1446.

Dinh, T. T. N., et al. (2010). Intramuscular fat and fatty acid composition of longissimus muscle from divergent pure breeds of cattle. *J.Anim.Sci.*, 88, 756-766.

Fernandez, X., Monin, G., Talmant, A., Mourot, J. and Lebret, B. (1999). Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of *M. longissimus lumborum*. *Meat Science*, 53, 67-72.

Fortin, A. (1986). Development of Backfat and Individual Fat Layers in the Pig and its Relationship with Carcass lean. *Meat Science*, 18, 255-270.

Furman, M., Malovrh, S., Levart, A. and Kovac, M. (2010). Fatty acid composition of meat and adipose tissue from Krskopolje pigsand commercial fatteners in Slovenia. *Archiv Tierzucht*, 53, 73-84.

Greenleaf, J. F. (1986). *Tissue characterization with ultrasound (Volume I)*. Florida: CRC Press, Inc.

Hustad, G. O., Richardson, T., Winder, W. C. and Dean, M. P. (1970). Factors Affecting Sound Velocity (Triggering Frequency) in Fats and Oils. *Journal of Dairy Science*, 53, 1525-1531.

Javanaud, C. and Rahalkar, R. R. (1988). Velocity of Sound in Vegetable Oils. *Fat Sci.Technol*, 90, 73-75.

Koch, T., Brand, S., Lakshmanan, S., Raum, K., Wicke, M. and Mörlein, D. (2010). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle. *Meat Science*, submitted.

Link, G. (2007). Beziehungen zwischen objektiv und subjektiv ermittelten Fleischqualitätsparametern unter besonderer Berücksichtigung der Muskelstrukturmerkmale im *Musculus longissimus* von Schweinen. In : Ph.D. Thesis, Department of animal science, Georg-August-University Göttingen.

Lo Fiego, D. P., Santoro, P., Macchioni, P. and De Leonibus, E. (2005). Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Science*, 69, 107-114.

Maleky, F., Campos, R. and Marangoni, A. G. (2007). Structural and Mechanical Properties of Fats Quantified by Ultrasonics. *J.Amer Oil Chem Soc*, 84, 331-338.

McClements, D. J. and Povey, M. J. W. (1988). Investigation of phase transitions in glycerine/paraffin oil mixtures using ultrasound velocity measurements. *J.Amer Oil Chem Soc*, 65, 1791-1795.

McClements, D. J. and Povey, M. J. W. (1992). Ultrasonic analysis of edible fats and oils. *Ultrasonics*, 30, 383-388.

Meyer, W., Neurand, K. and Radke, B. (1982). Collagen fibre arrangement in the skin of the pig. *J.Anat.*, 134, 139-148.

Miles, C. A. and Fursey, G. A. (1977). Measurement of the fat content of meat using ultrasonic waves. *Fd.Chem.*, 2, 107-118.

Monziols, M., Bonneau, M., Davanel, A. and Kouba, M. (2007). Comparison of the lipid content and fatty acid composition of intramuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science*, 76, 54-60.

Moody, W. G. and Zobrisky, S. E. (1966). Study of backfat layers of swine. *J.Anim.Sci.*, 25, 809-813.

Mörlein, D., Rosner, F., Brand, S., Jenderka, K. V. and Wicke, M. (2005). Non-destructive estimation of the intramsucular fat content of the *longissimus* muscle of pigs by means of spectral analysis of ultrasound echo signals. *Meat Science*, 69, 187-199.

Müller, S. and Polten, S. (2004). Vergleichsuntersuchung zur Ultraschall-Speckdickenmessung beim Schwein im Rahmen der Eigenleistungprüfung. *Arch.Tierz., Dummersdorf*, 47, 249-263.

Ninoles, L., Clemente, G., Ventanas, S. and Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Ninoles, L., Sanjuan, N., Ventanas, S. and Benedito, J. (2008). Ultrasonic and sensory characterization of dry-cured ham fat from Iberian pigs with different genetics and feeding backgrounds. *Meat Science*, 80, 869-902.

Olerud, J. E., et al. (1987). Ultrasonic Assessment of Skin and Wounds With the Scanning Laser Acoustic Microscope. *J.Invest.Dermatol.*, 88, 615-623.

Scheeder, M. R. L., Gläser, K. R., Eichenberger, B. and Wenk, C. (2000). Influence of different fats in pig feed on fatty acid composition of phospholipids and physical meat quality characteristics. *Eur.J.Lipid Sci.Tech.*, 102, 391-401.

Smith, N. B. (1996). Effect of myofibril length and tissue constituents on acoustic propagation properties of muscle. *Ph.D.Thesis, University of Illinois.*

Villegas, F. J., Hedrick, H. B., Veum, T. L., McFate, K. L. and Bailey, M. E. (1973). Effect of diet and breed on fatty acid composition of porcine adipose tissue. *Journal of Animal Science*, 36, 663-668.

Wood, J. D. (1973). The fatty acid composition of backfat from Pietrain and Large White pigs. *Anim.Prod.*, 17, 281-285.

Wood, J. D., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343-358.

Wood, J. D., Enser, M., Whittington, F. M., Moncrieff, C. B. and Kempster, A. J. (1989). Backfat Composition in Pigs: Differences Between Fat Thickness Groups and Sexes. *Livestock production science*, 22, 351-362.

Wood, J. D., et al. (2004). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, 67, 651-667.

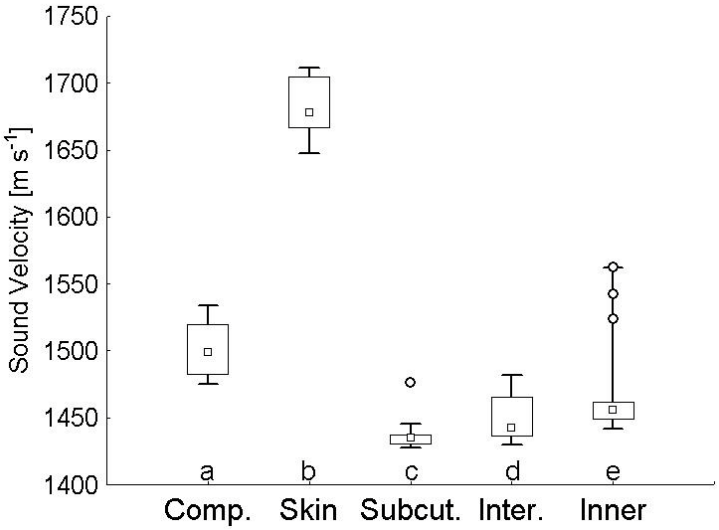


Figure 1: Variation of sound velocity of backfat samples determined by 10-MHz ultrasound.
[a,b,c,d,e]: different letters indicate significant differences between layers (F = 612.47)
Compound = all backfat layers and skin (n = 26); skin (n = 21);
subcutaneous fat layer (n = 27); intermediate fat layer (n = 23); inner fat layer (n = 17).
The outliers exhibit extreme amounts of fatty acids and dry matter.

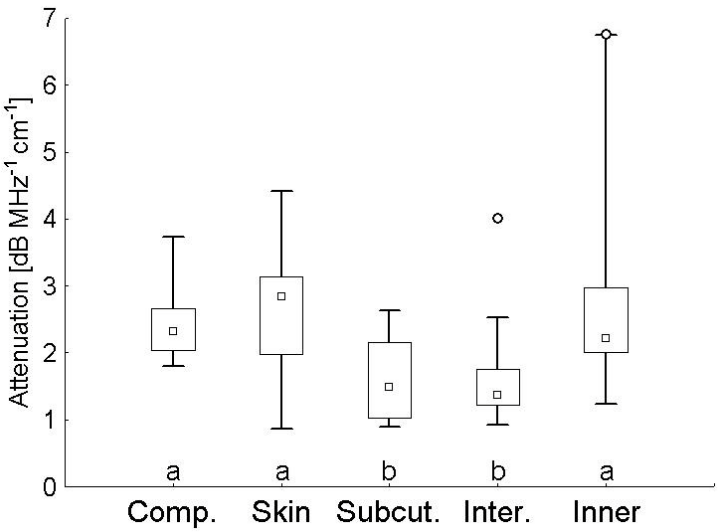


Figure 2: Variation of attenuation of backfat samples determined by 10-MHz ultrasound.
[a,b]: different letters indicate significant differences between layers (F = 8.09)
Compound = all backfat layers and skin (n = 26); skin (n = 21);
subcutaneous fat layer (n = 27); intermediate fat layer (n = 23); inner fat layer (n = 17).
The outliers exhibit extreme amounts of fatty acids and dry matter.

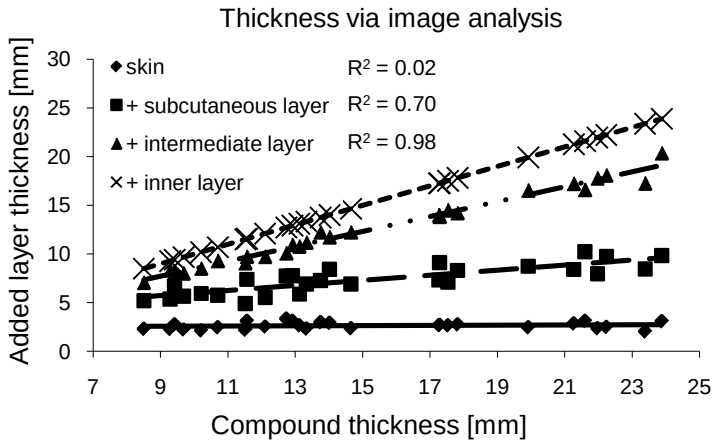


Figure 3: Linear regression analysis of the cumulative thickness values of the individual layers (skin + fat layers 1 to 3) versus compound thickness determined by optical image analysis (n=26).

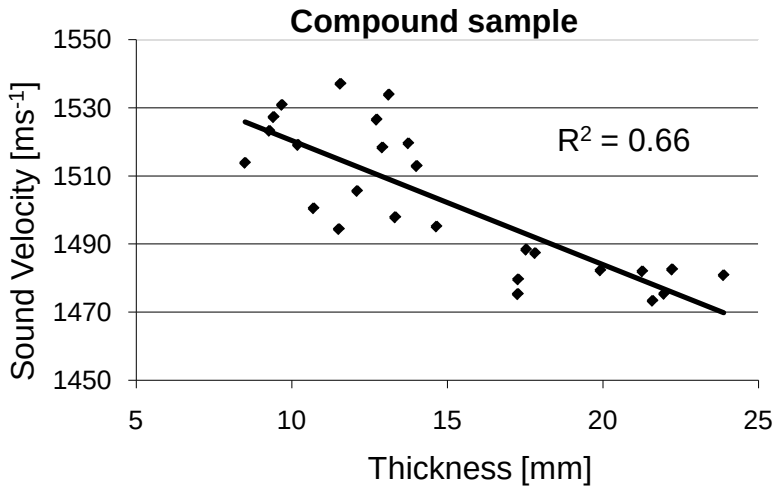


Figure 4: Linear regression analysis of the sound velocity versus compound thickness (fat layers + skin) determined by image analysis (n=26).

Table 1: Thickness and relative dry matter content (n=27), and fatty acid composition (n=20) of individual backfat layers (means ± standard deviations). Significant differences between the fat layers are indicated by different superscript letters.

	F-value	Subcutaneous layer	Intermediate layer	Inner layer
Thickness [mm]	13.17	4.72 ± 1.36 [a]	5.15 ± 2.60 [a]	2.74 ± 1.24 [b]
Dry matter [%]	15.95	88.69 ± 2.03 [a]	88.76 ± 3.68 [a]	83.30 ± 5.54 [b]
Fatty acids [%]	0.68	77.10 ± 3.26	74.67 ± 8.35	75.54 ± 7.29
SFA [%]	21.57	34.73 ± 2.68 [a]	39.05 ± 2.79 [b]	39.89 ± 2.53 [b]
MUFA [%]	3.03	47.02 ± 2.60 [a]	45.10 ± 2.59 [b]	45.80 ± 2.29 [b]
PUFA [%]	7.12	18.26 ± 3.44 [a]	15.85 ± 3.53 [b]	14.31 ± 3.02 [b]
C14:0 (Myristic) [%]	0.59	1.05 ± 0.25	1.00 ± 0.22	0.98 ± 0.16
C16:0 (Palmitic) [%]	5.53	19.53 ± 1.46 [a]	20.84 ± 1.50 [b]	20.79 ± 1.27 [b]
C16:1 (Palmitoleic) [%]	3.61	1.73 ± 0.41 [a]	1.44 ± 0.34 [b]	1.47 ± 0.36 [b]
C18:0 (Stearic) [%]	25.10	13.77 ± 1.73 [a]	16.74 ± 1.81 [b]	17.70 ± 1.95 [b]
C18:1 (Oleic) [%]	3.58	43.32 ± 2.26 [a]	41.49 ± 2.26 [b]	42.41 ± 1.97 [ab]
C18:2 (Linoleic) [%]	6.61	13.42 ± 2.91 [a]	11.31 ± 3.07 [b]	10.18 ± 2.58 [b]
C18:3 (Linolenic) [%]	5.61	1.17 ± 0.34 [a]	0.94 ± 0.30 [b]	0.85 ± 0.31 [b]
C20:2 (Eicosanoic) [%]	0.48	2.71 ± 0.40	2.84 ± 1.21	2.53 ± 0.30

SFA: saturated fatty-acids, MUFA: mono-unsaturated fatty-acids, PUFA: poly-unsaturated fatty-acids

Table 2: Pearson product moment correlations between carcass characteristics and chemical composition of the individual backfat layers.

	weight	LMP	BW	MW	IMF	thickness
Subcutaneous fat layer (n=20)						
Fatty acids	0.21	-0.62 **	0.62 **	-0.19	0.42	0.57 **
Dry matter	0.23	-0.40 *	0.43 *	0.02	0.21	0.31
SFA	0.07	-0.39	0.34	-0.01	0.34	0.18
MUFA	0.10	-0.12	0.14	-0.08	0.30	0.15
PUFA	-0.13	0.39	-0.36	0.07	-0.49 *	-0.25
Intermediate fat layer (n=20)						
Fatty acids	0.37	-0.72 ***	0.85 ***	-0.08	0.34	0.81 ***
Dry matter	0.36	-0.66 ***	0.72 ***	-0.11	0.22	0.82 ***
SFA	0.15	-0.37	0.37	0.03	0.24	0.37
MUFA	-0.07	-0.12	0.11	-0.12	0.17	0.04
PUFA	-0.07	0.37	-0.37	0.07	-0.32	-0.33
Inner fat layer (n=20)						
Fatty acids	0.37	-0.73 ***	0.73 ***	-0.28	0.59 **	0.70 ***
Dry matter	0.43 *	-0.39	0.45 *	0.19	0.16	0.47 *
SFA	0.21	-0.46 *	0.48 *	0.03	0.14	0.48 *
MUFA	-0.12	-0.09	0.01	-0.20	0.24	-0.08
PUFA	-0.08	0.45 *	-0.42	0.13	-0.31	-0.34

SFA: saturated fatty-acids, MUFA: mono-unsaturated fatty-acids, PUFA: poly-unsaturated fatty-acids; weight: hot carcass weight, LMP: lean meat percentage, BW: backfat width, MW: muscle width, IMF: intramuscular fat, thickness: thickness of the individual layers determined with image analysis

*p<0.05; **p<0.01; ***p<0.001

Table 3: Pearson product moment correlations between carcass characteristics and ultrasound parameters of skin, compound sample and the individual fat layers.

	weight	BW	IMF	thickness
	Compound (n = 25)			
Sound velocity	-0.26	-0.80 ***	-0.27	-0.81 ***
Attenuation	-0.12	-0.26	0.03	-0.31
	Skin (n = 20)			
Sound velocity	0.29	0.28	-0.01	0.13
Attenuation	-0.35	-0.27	0.45 *	0.17
	Subcutaneous fat layer (n = 26)			
Sound velocity	-0.12	-0.22	-0.32	-0.27
Attenuation	0.11	-0.02	-0.25	-0.09
	Intermediate fat layer (n = 22)			
Sound velocity	-0.31	-0.58 **	-0.08	-0.61 **
Attenuation	-0.04	-0.36	0.10	-0.44 *
	Inner fat layer (n = 17)			
Sound velocity	-0.47	-0.44	-0.33	-0.57 *
Attenuation	-0.05	0.01	-0.14	-0.11

weight: hot carcass weight, BW: backfat width, IMF: intramuscular fat,
thickness: thickness of the individual layers determined with image analysis;
n: differences in the amount of samples are reasoned in insufficient measurements
*p<0.05; **p<0.01; ***p<0.001

Table 4: Pearson product moment correlations between chemical compositions and ultrasound parameters.

	FA	DM	SFA	MUFA	PUFA
	Subcutaneous fat layer (n = 20)				
Sound velocity	-0.46 *	-0.42 *	-0.08	-0.24	0.24
Attenuation	0.03	-0.31	-0.02	-0.54 *	0.42
	Intermediate fat layer (n = 17)				
Sound velocity	-0.53 *	-0.65 *	-0.15	0.00	0.12
Attenuation	-0.22	-0.36	0.08	-0.34	0.19
	Inner fat layer (n = 14)				
Sound velocity	-0.46	-0.01	-0.11	-0.17	0.25
Attenuation	0.09	-0.16	0.11	-0.29	0.12
	Combined fat layers (n = 51)				
Sound velocity	-0.37 **	-0.39 **	0.29 *	-0.14	-0.18
Attenuation	0.04	-0.37 **	0.25	-0.24	-0.08

FA: total amount of fatty acids, DM: dry matter, SFA: saturated fatty-acids,
MUFA: mono-unsaturated fatty-acids, PUFA: poly-unsaturated fatty-acids
n: differences in the amount of samples are reasoned in insufficient measurements
*p<0.05; **p<0.01

Paper III:

Lakshmanan, S.; Koch, T.; Brand, S.; Männicke, N.; Wicke, M.; Mörlein, D. and Raum, K.

Prediction of the intramuscular fat content in loin muscle of pig carcasses by quantitative time-resolved ultrasound

Meat Science

Submitted: 15. December 2010

PREDICTION OF THE INTRAMUSCULAR FAT CONTENT IN LOIN MUSCLE OF PIG CARCASSES BY QUANTITATIVE TIME-RESOLVED ULTRASOUND

S. LAKSHMANAN*[x]; T. KOCH[‡x]; S. BRAND[#]; N. MÄNNICKE[*]; M. WICKE[‡]; D. MÖRLEIN[‡] and K. RAUM*[†]

[*]Julius Wolff Institute & Berlin – Brandenburg School for Regenerative Therapies

Charité – Universitätsmedizin Berlin, Germany

[‡]Department of Animal Sciences, Georg August University Göttingen, Germany

[#]Fraunhofer Institute of Material Mechanics, Halle, Germany

[x] Both authors equally contributed to this investigation.

[†]Corresponding Author

Prof. Dr.rer.nat. Kay Raum

Julius Wolff Institute & Berlin – Brandenburg School for Regenerative Therapies,

Charité – Universitätsmedizin Berlin

Augustenburger Platz 1, 13353 Berlin, Germany

Phone: +49 30 450 539 503

Fax: +49 30 450 539 952

e-mail: Kay.Raum@charite.de

The aim of the present study was to non-invasively predict the IMF of the M. *longissimus* on pig carcasses analysing the backscattered ultrasound signal. Incorporation of IMF in the connective tissue is known to modify its structure and elastic properties. Thus, IMF is expected to affect the ultrasound backscatter properties of muscle. A modified ultrasound device with a centre frequency of 2.7 MHz was used and improved ultrasound pre-processing algorithms were applied to allow accurate estimation of acoustic parameters.

Data acquisition was performed on 82 warm carcasses with up to 3 measurements each. Linear correlations with IMF ranged up to r =.61 for attenuation. Multiple linear regression resulted in an R^2 value of .76 (prediction error = 0.34 %). Considering the IMF variation within the muscle and the small region for IMF estimation, the achieved prediction errors seem reasonable. Further improvements could be expected measuring varying positions of the muscle. Furthermore, data-evaluation in real-time is required to detect erroneous measurements.

Key Words - Intramuscular fat, Muscle, Ultrasound, Spectrum, Cepstrum, Apparent Integrated Backscatter, Integrated cepstrum

1. Introduction

The intramuscular fat content (IMF) is widely regarded as one of the major parameters influencing quality and sensory characteristics of meat (Fernandez, Monin, Talmant, Mourot & Lebret, 1999; Suzuki, Irie, Kadowaki, Shibata, Kumagai & Nishida, 2005; Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes & Whittington, 2008). These relations are considered not to be linear and threshold levels between 2 and 3.5 % IMF in porcine *longissimus* muscle at the 2^{nd} / 3^{rd} last rib have been proposed as benchmarks for optimal taste (Fernandez et al., 1999; Blanchard, Warkup, Ellis, Willis & Avery, 1999; Kipfmüller, Bodis, Peschke & Eichinger, 2000). However, recent investigations on German pig populations revealed average IMF values of about 1 to 1.5 %, indicating that only a minority of the animals actually reach the proposed minimum IMF threshold level of 2 % (Mörlein, Link, Werner & Wicke, 2007).

For the establishment of a sensory quality based marketing system, the classification of meat according to the IMF levels is indispensable. Ultrasound (US) has been demonstrated to be promising for prediction of IMF in a fast, inexpensive and non-destructive way. Previous studies have either measured the sound velocity of the acoustic signal (Park,

Whittaker, Miller & Hale, 1994; Benedito, Carcel, Rossello & Mulet, 2001) or have analysed the texture of the gray scale backscatter images (Brethour, 1994; Hassen, Wilson, Amin, Rouse & Hays, 2001). Although these methods have been quite successful in the prediction of IMF on beef in vivo or hot carcasses with R^2 values up to .75 (Whittaker, Park, Thane, Miller & Savell, 1992; Hassen et al., 2001; Hassen, Wilson & Rouse, 2003), most studies performed in porcine muscle were less predictive ($R^2 \leq .4$) (Ville, Rombouts, Van Hecke, Perremans, Maes, Spincemaille & Geers, 1997; Newcom, Baas & Lampe, 2002). Recently, ultrasound measurements on living pigs resulted in R^2 values of .48 predicting the chemically determined IMF via image analysis (Maignel, Daigle, Gariépy, Wilson & Sullivan, 2010).

During the last decade, spectral analysis of the backscattered ultrasound signals has been proven to provide more detailed information about tissue constitution compared to conventional image texture or sound velocity analyses (Park, Whittaker, Miller & Bray, 1994; Lizzi, Feleppa, Alam & Deng, 2003). One major advantage of spectral analysis of the radio frequency (RF) backscatter signals is that it contains quantitative information about composition and elastic properties of structures that are comparable to or smaller than the acoustic wavelength. The frequency dependence of acoustic backscatter can therefore be used to quantify structural dimensions that are not visible in the ultrasound image or to differentiate between tissue types (Raju and Srinivasan, 2001; Scheipers, Ermert, Sommerfeld, Garcia-Schürmann, Senge & Philippou, 2003; Banihashemi, Vlad, Debeljevic, Giles, Kolios & Czarnota, 2008; Gelse, Olk, Eichhorn, Swoboda, Schoene & Raum, 2010). In muscle tissue, the major acoustic inhomogeneities are considered to be the connective tissue interfaces between adjacent muscle fibres and between muscle bundles (Lizzi et al., 2003). Alterations of this structure are considered to affect the backscatter spectrum. Lizzi (1997) has introduced several spectral parameters that are related to the structure of the scatterers. While the slope of the obtained power spectrum *m* (dB/MHz) is related to the size, midband fit M (dB, value of the linear fit at the center frequency) and spectral intercept *I* (dB, extrapolated amplitude value at 0 MHz) are affected by size, concentration, and impedance of the scatterers (Lizzi, Astor, Liu, Deng, Coleman & Silverman, 1997; Lizzi, Alam, Mikaelian, Lee & Feleppa, 2006). Another common spectral estimate is the apparent integrated backscatter amplitude *AIB* (dB, power spectrum within the bandwidth of the transducer) (Gelse et al., 2010). Tissue boundaries that are separated at distances larger than the wavelength and the spatial resolution limit of the imaging system, e.g. the marbling structure in muscle, can often be seen directly in the US gray scale images. A more precise estimation of distance distributions along the

sound propagation path from RF signals however, can be obtained using cepstral analysis (Oppenheim and Schafer, 2004).

The composition of connective tissue is influenced by multiple parameters, e.g. age or breed. Moreover, IMF is known to alter the connective tissue structure due to the deposition of fat inside the perimysium, i.e. between the primary muscle fiber bundles (Essen-Gustavsson, Karlsson, Lundström & Enfält, 1994). This deposition is associated with a partial destruction of the honeycomb structure of the connective tissue, which in turn reduces mechanical strength and shear modulus of the tissue (Nishimura, Hattori & Takahashi, 1999; Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard & Enser, 2003) and explains the association with sound velocity. On the other hand, the acoustic properties of fat are remarkably different from those of muscle and connective tissue. Therefore, an increasing amount of fat deposited between muscle fibers and fiber bundles is hypothesized to alter the characteristics of the backscatter power spectrum and to increase the amplitude of reflections at muscle bundle boundaries. Moreover, an increase of backscatter and reflections amplitudes should result in an increase of the attenuation in muscle (Smith, 1996).

Yet, only few studies have targeted the use of spectral analysis to predict the IMF content of bovine (Park et al., 1994; Abou El Karam, Buquet, Berge & Culioli, 1997) and porcine muscle (Mörlein, Rosner, Brand, Jenderka & Wicke, 2005). The latter have performed analysis of unprocessed backscatter signals obtained with a medical diagnostic ultrasound device to estimate the intramuscular fat content of porcine loin muscle. Although promising results were obtained (root mean squared error of prediction RMSEP = 0.36 %), the instrument and the prediction errors were not yet satisfying for industrial use at slaughter.

A prerequisite of a reliable ultrasound spectral analysis is an exact knowledge of the sound properties at the region of interest (ROI) and all tissues passed by the RF signal. These properties are determined by system specific effects, e.g. center frequency, bandwidth and sound field produced by the transducer. Furthermore, refraction and attenuation in all tissues along the sound propagation path will influence the sound field geometry (Gärtner, 2001). The incorporation of sophisticated calibration and correction methods are therefore anticipated to improve the accuracy of ultrasound based IMF predictions (Brand, Mörlein & Rosner, 2002). Thus, reference data of porcine skin, backfat and muscle, collected under laboratory conditions (Koch, Brand, Sannachi, Raum, Wicke & Mörlein, 2010a; Koch, Brand, Sannachi, Raum, Wicke & Mörlein, 2010b) were implemented into the correction algorithms

used during the present study. The IMF was thereafter predicted from backscatter data collected with a customized commercial hand-held US device.

2. Materials and methods

2.1. Animals

A representative variation of meat properties was obtained by conducting three series of data acquisitions at different German slaughter houses. For all measurements left carcass sides were used. 54 animals (commercial crossbreds) were investigated at commercial slaughter facilities and selected according to 3 levels (low, mid, high) of carcass weight and lean meat percentage (LMP) each. In addition, 28 animals (Duroc and Duroc-crossbreds) raised and slaughtered at an experimental device were measured to increase the range of the intramuscular fat. After evisceration, muscle width *MW* (mm) and backfat width *BW* (mm) were determined with a carcass grading probe (Fat-o-Meat'er, Carometec, Denmark) on the German official site of carcass classification (between 2^{nd} and 3^{rd} last rib, 7 cm off the carcass split line).

2.2. Ultrasound data acquisition

The ultrasound readings were made on the same location as the Fat-o-Meat'er measurements 45 min post mortem (p.m.) on suspended carcasses including skin and subcutaneous fat layers (Fig. 1). At this time the muscle temperature was approximately 38°C. Up to three replicate readings were performed parallel to the split line. For each measurement the ultrasound device was detached and re-positioned. Care was taken to ensure comparability in terms of measurement conditions (e.g. time p.m., position). Acoustic coupling was achieved using ultrasound contact gel.

2.3. Chemical IMF determination

Approximately 24 h p.m., after chilling of the carcasses over night, a chop was excised from the *m. longissimus* at the $2^{nd}/3^{rd}$ last rib for subsequent laboratory analyses. IMF was determined in duplicate on homogenized samples after removal of the subcutaneous fat.

The homogenization was done using a Grindomix GM200 blender (Retsch GmbH; Haan, Germany) at 6000 rpm for 30 seconds. IMF was extraced with petroleum ether using a Soxtherm-apparatus after HCl pre-treatment according to German Food Legislation (LFGB, 2005) and is given related to fresh matter. The average standard deviation of duplicate IMF determination is 0.05 % IMF.

2.4. Ultrasound data analysis

For ultrasound readings, a customized carcass grading device Ultrafom300 (Carometec, Denmark) was used. The original device consists of a linear array of 64 unfocused transducer elements (single element size: $x = 18$ mm; $y = 0.8$ mm) aligned along the *y*-axis. The nominal centre frequency of the elements is 3.2 MHz. Single elements are excited separately and produce an elliptically shaped sound field with the focus at a distance of approximately 100 mm. The beam in the focal plane has -6 dB extensions of 2.5 mm and 50 mm in the *x*- and *y*- directions, respectively. This configuration has been optimized to measure the acoustic reflections at the muscle rib interface over an area of approximately 2.5 mm x 100 mm (with the long dimension parallel to *y*-axis and the carcass split line). It should be noted that this system was not designed for imaging but for detection of the fat-muscle and muscle-rib boundaries.

The near field, i.e. the range from the transducer surface to the focal plane, is characterized by an inhomogeneous pressure distribution, rendering a quantitative backscatter analysis impossible. Therefore, an acoustic lens was designed that shifts the focus of the sound field towards the muscle region and produces smooth spatial and spectral characteristics within the muscle region of interest. The necessary radius of curvature (ROC) of the lens was computed using the Field II simulation software (Jensen and Svendsen, 1992). The lens was casted using silicone rubber (Elasosil M4641, Wacker Chemie AG, Munich, Germany) mixed with aluminium oxide particles (30 wt%; mean diameter: 5 µm). A time dependent amplification of the pulse echo signal was realized by an external function generator (HM 8150, HAMEG Instruments GmbH, Mainhausen, Germany). A linearly increasing ramp function ensured a good signal-to-noise ratio over the entire depth down to the muscle/rib boundary. With these hardware modifications, the measured centre frequency of a pulse reflected at a plane reflector in the focal plane was 2.7 MHz with a -6 dB bandwidth from 2.1 – 3.5 MHz. The other spatial, temporal and spectral sound field

characteristics for measurements in water at 38 °C with and without the lens are summarized in Fig. 2 and Table 1 (Raum and O'Brien, 1997).

The pulse-echoes of all 64 elements were digitized using an A/D converter with a sampling frequency of 10 MHz and 8 bit resolution in a pulse echo time interval of 153 µs and stored on a personal computer for offline processing. All subsequent data processing steps were performed with custom functions using Matlab R2008a® (Mathworks Inc., Natick, USA).

2.5 Ultrasound data pre-processing

The correction steps applied to the time-gated pulse-echo signals prior to the spectral and cepstral parameter estimations are described in the following sections.

2.5.1. Time-gain compensation (TGC)

The time-dependent amplification was compensated to ensure a uniform amplification within the entire focus range.

2.5.2. Sound field correction (SFC)

The signal amplitude of reflected and backscattered waves varies with respect to the distance from the focal plane. A time of flight based defocus correction was used to calibrate the system in water at 37 °C (Raum, Reisshauer & Brandt, 2004; Raum, Kempf, Hein, Schubert & Maurer, 2007; Raum, 2008). Briefly, the reflections of a plane 4-wt% agar reflector were recorded at successively increasing distances to the transducer. Mass density and sound velocity in the agar phantom were $\rho_{agar} = 1.003$ gcm^{-3} and $v_{agar} = 1542$ ms^{-1}, respectively. The measured confocal reflection amplitude was estimated to be 35 dB smaller than that of a perfect reflector.

Because of differences of attenuation and sound velocities in skin, backfat and muscle compared to that in water, the focus position is shifted in the carcass (Fig. 3). To compensate for this effect, a refraction correction based on the average sound velocities (table 2) and thicknesses in the skin-backfat compound and in muscle has been applied (Lakshmanan,

Koch, Mörlein, Brand & Raum, 2010). However, the used measurement conditions (e.g. tissue temperature or transducer frequency range) of available data widely differ affecting the reported attenuations and sound velocity values. Therefore, reference data of muscle and backfat were obtained with a scanning acoustic microscope under close-to hot carcass conditions (Koch et al., 2010a; Koch et al., 2010b). The obtained results are summarised and compared to literature data in table 2. The backfat width was estimated from the time of flight to the backfat-muscle boundary TOF_{BF}. In a companion in-vitro study of a subset (Koch et al., 2010b) the velocity in the skin-backfat compound was found to be strongly related to the thickness ratio of the two tissue types and that the compound velocity can be estimated using the empirical relation:

$$\nu_{BFcompound} = 1555 - 3.94 mm^{-1}\ ms^{-1} \cdot BW\ . \quad (1)$$

BW and $\nu_{BFcompound}$ were estimated from TOF_{BF} using an iterative minimization algorithm.

The average sound velocity in muscle ν_{muscle} has been found to be 1620.5 ± 4.6 ms^{-1} within the evaluated carcasses (Koch et al., 2010a). A weak correlation with IMF (R^2 = .13; RMSE = 4.33 ms^{-1}) was also observed in that study. This variation had no remarkable effect on the refraction correction. Therefore, only the mean velocity was included in the correction model.

The distance z from the transducer of a gated signal within the muscle was estimated using the relation:

$$z_{\text{muscle}} = BW + (TOF_{Gateposition} - TOF_{BF}) \times \nu_{muscle} / 2\ . \quad (2)$$

It can be seen in Fig. 3 that the axial intensity distribution in the backfat muscle compound is shifted towards the transducer compared to that in water. The Field II simulations were used to estimate the axial shift. Then the pulse echo measured in water at z_{ref} that exhibits the same decrease of intensity (relative to the focal plane) as that estimated for z_{muscle} was used as a reference signal at $TOF_{Gateposition}$ for further calculations.

2.5.3. Wave front curvature compensation (WFCC)

Due to the curvature of the wave front of the focused sound field reflections of a plane reflector exhibit a further phase cancellation with increasing distance from the focal plane. Therefore, the decrease of the measured amplitude of a plane reflector with respect to the defocus distance is larger than that measured from backscattered signals collected at the corresponding time gates. This amplitude deviation between signals reflected from a plane agar reflector after SFC correction and those backscattered within the corresponding time gate $TOF_{Gateposition}$ was assessed by measurements in a tissue mimicking agar phantom. This phantom was made of graphite powder immersed in agar, as described by (Madsen, Dong, Frank, Garra, Wear, Wilson, Zagzebski, Miller, Shung, Wang, Feleppa, Liu, O'Brien, Jr., Topp, Sanghvi, Zaitsev, Hall, Fowlkes, Kripfgans & Miller, 1999). The sound velocity and attenuation are listed in Table 2. The phantom was measured using the same device settings as for the muscle measurements and it was attached directly to the transducer surface. Figure 4 shows the apparent integrated backscatter amplitude *AIB* as a function of the defocus distance. For a proper sound field correction, *AIB* is supposed to decrease linearly with increasing depth. Due to the curvature of the focused beam before and after the focal plane, the estimated *AIBs* increased with increasing distance from the focal plane (Fig. 4a).

It can be seen in Fig. 4b that after SFC and WFCC corrections the measured *AIB* in the focal range only depends on the attenuation of the phantom.

2.5.4. Attenuation compensation (AC)

The sound waves are attenuated on the two-way travel from the transducer surface to the muscle region of interest. Further losses arise from partial reflections at tissue boundaries. Refraction of the sound field and attenuation after propagation through skin and backfat were estimated using sound velocity and attenuation values assessed in a previous study (Koch et al., 2010b). The muscle attenuation α_{muscle} within the ROI was estimated using a sliding window technique as proposed by (Bigelow and O'Brien, 2005; Bigelow, Oelze & O'Brien, 2005). For this, all 64 spectra were averaged for each $TOF_{Gateposition}$ and the slope of the frequency dependent attenuation was assessed by linear regression. Finally, the attenuation of the overlaying backfat tissue α_{BF} was compensated. For this, the mean value of α_{BF} = 2.1 $dBMHz^{-1}cm^{-1}$ assessed in a previous study was used (Koch et al., 2010b).

2.6. Spectral parameter estimation

Backscatter properties of muscle tissue were assessed within a ROI 54 mm x 15 mm (i.e. the axial -6 dB range from 45 to 59 mm in water). The normalized power spectra *S(f)* were calculated after the above mentioned corrections as follows.

First, a part of the signal was gated using a sliding Hanning window. The positions of the first and last windows were placed 10 mm above and 7 mm below the estimated focus position, respectively. The gate width was equivalent to the 2-fold pulse duration (3 µs), which corresponds to a depth range of ~ 15 mm assuming a sound velocity of 1620 ms^{-1} in muscle. The overlap between adjacent gate windows was set to 50 %. The logarithmic power spectrum was normalized to the reference spectrum obtained from the plane agar reflector, after SFC, WFCC and AC correction. Spectra intensity within the frequency bandwidth below -40 dB (close to noise level) were excluded. The excluded RF signal percent (RF_{excl}) was also stored indicating the quality of the acquired data. The remaining normalized spectra were averaged within the entire ROI. AIB was calculated in the frequency range between 2.1 MHz and 3.5 MHz:

$$AIB = \frac{1}{\Delta f}\int S(f)\,df \tag{3}$$

Midband fit *M* and spectral slope *m* were calculated as described elsewhere (Lizzi et al., 2003) and illustrated in Fig. 5.

2.7. Cepstral parameter estimation

The power cepstral data analysis can be described as the Fourier transform (FT) of the power spectral density of a time signal:

$$C(\tau) = \left|FT\left(log_{10}|S_{muscle}(f)|^2 - log_{10}|S_{ref}(f)|^2\right)\right|^2 \tag{4}$$

where $S_{muscle}(f)$ and $S_{ref}(f)$ are the Fourier transforms of the time-gated backscatter signals of a the muscle and the reference, respectively. Cepstral peaks are associated with the occurrence and time delay of several echoes within the time gate. Further restrictions are that the time delay should be larger than the pulse length, and the analysis is only meaningful within the spectral bandwidth of the ultrasound system. The window length was 5 times of the pulse duration and the overlap was set to 90 %. Only the SFC and WFCC corrections have

been applied for the cepstral analysis. Prior to the FT the difference spectrum was preconditioned by removing DC and linear components. Similarly to the spectral analysis, signals with cepstral amplitudes below -40 dB were excluded. The cepstral first peak intensity value $C_{fp}(\tau_{fp})$, the corresponding time-delay τ_{fp} and its standard deviation were determined by fitting averaged cepstrum by Weibull function. The integrated cepstrum *IC*:

$$IC = \frac{1}{\Delta\tau}\int C(\tau)d\tau \tag{5}$$

within the interval $\Delta\tau$ from 0.6 µs – 1.6 µs were calculated from the averaged cepstra (Fig. 6).

The proper selection of the evaluation regions, the measurements, and the analysis of the data has been performed by a team of three ultrasounds experts (SL, NM, and KR).

2.8. Statistical analysis

Statistical analysis was performed using SAS 9.1 (SAS Institute, Cary, USA), STATISTICA 7.1 (StatSoft, Tulsa, USA) and The Unscrambler 9.2 (CAMO AS, Oslo, Norway). Linear correlations between acoustic parameters and the IMF were computed as Pearson product-moment correlations. Multiple linear regression (MLR) was performed to model the IMF. Acoustic parameters were included into the model when the required level of significance reached $p < .05$. Full-cross validation was applied. Thus, every sample was left out once to test the model based on the remaining samples. Correspondingly, model R^2 and root-mean square error of prediction (RMSEP) are given. For classification purposes it was analysed, whether the carcasses were correctly assigned to 3 classes (IMF_{chem}) based on predicted fat content (IMF_{US}). The thresholds were chosen as follows: < 1 % IMF (LOW), between 1 and 2 % IMF (MID) and > 2 % IMF (HIGH).

All results were considered significant for $p < .05$.

3. RESULTS

Prior to the statistical analysis, all measurements were checked for data quality. Out of the 218 performed ultrasound data acquisitions (54 x 3 and 28 x 2), 83 measurements (38 %) had to be discarded. The reasons for excluding the data were: i) focus area and corresponding

ROI at the muscle-rib interface (8 %), ii) very weak scattering amplitudes resulting in an insufficient SNR (9 %), iii) multiple reflections of the backfat interfaces in the muscle ROI due to bad coupling (4 %), iv) AIB estimation > -20 dB due to higher pressure given on skin surface during measurement, v) bad coupling of the transducer (12 %). For 20 out of 82 carcasses all three US readings had to be excluded. For the remaining carcasses, the estimated US parameters were averaged per carcass and used for further analysis.

The estimated parameters are summarised in Table 3. The corresponding descriptive statistics are shown in Table 4. The IMF of the loin samples under investigation ranged from 0.65 – 3.56 % (1.53 ± 0.69 %) and is shown in more detail in figure 7.

3.1 Correlations between US parameters and IMF

The linear correlations between the US parameters and the IMF are given in Table 5. Moderate correlations of IMF with attenuation (α_{muscle}) and spectral amplitudes (*AIB* and *M*) were observed. Furthermore, slight positive relationships could be found between the IMF content and TOF_{BF}. Weak correlations were observed between the cepstral parameters and IMF.

3.2 Multivariate IMF prediction

Multiple linear regression analysis (MLR) was performed to predict the IMF using the ultrasound parameters. A highly significant model (R^2 = .76; RMSEP = 0.34 %) could be obtained by a combination of backfat compound time of flight TOF_{BF}, muscle attenuation α_{muscle} and spectral slope *m* (Eq. 6):

$$IMF_{US} = -2.416\ \% + (0.108\ \mu s^{-1} TOF_{BF})\% + (4.755 dB^{-1} MHz.cm\ \alpha_{muscle})\% - (0.457\ m\ dB^{-1} MHz)\% \quad (6)$$

Including other carcass characteristics (e.g. hot carcass weight or backfat width) did not significantly improve the model. It should be mentioned that, even if α_{muscle} was the most important variable, the removal of even one of the 3 variables resulted in a tremendous increase of the prediction error (to at least 0.52 % IMF).

A comparison between IMF_{US} and IMF_{chem} is shown in figure 8. For 59 out of 62 analyzed carcasses the difference between the two prediction methods was smaller than 0.67 % IMF. The prediction error does not depend on the IMF level. Furthermore, about half of the samples could be predicted with a RMSEP of 0.2 % IMF or less.

The results of the classification into for meat quality important IMF groups of < 1 % (LOW), 1-2 % (MID) and > 2 % IMF (HIGH) and the comparison with the IMF_{chem} values is summarised in table 6. Overall, about 73 % of all samples were assigned to the correct IMF class. Furthermore, it should be mentioned that only 1 sample was falsely classified into the HIGH class. Using a single threshold (2 % IMF), about 92 % of all carcasses were correctly classified in LOW or HIGH IMF.

4. DISCUSSION

The carcass traits are mainly in good agreement with earlier investigations of German pig populations (Mörlein et al., 2007). The selection of carcasses based on LMP and carcass weight and the inclusion of Duroc and Duroc-crossbreds resulted in a high variability of IMF. Overall, the carcasses can be considered to represent the majority of commercially slaughtered pigs in Germany with a slightly higher proportion of carcasses with IMF values above 2 %.

4.1 Correlations between US parameters and IMF

Increasing IMF was previously mentioned to be related with increased attenuation in both, beef (Smith, 1996) and porcine muscle (Mörlein et al., 2005). Furthermore, earlier investigations performed on a subset (n=27) with a scanning acoustic microscope also revealed positive relationships (r = .66) between muscle attenuation of small excised samples and IMF of the corresponding loin chop (Koch et al., 2010a). Few studies report relations between IMF and spectral or cepstral parameters. Mörlein (2005) analysed a wide range of parameters obtained with a medical ultrasound device. Backscatter parameters (AIB, M) increased with IMF (r = .2 to r = .3). This is confirmed by the present study (r = .56). While Mörlein (2005) stated no significant relationships between IMF and cepstral parameters, the present study reports correlations up to r = .29. Procedural differences (e.g. the used frequency or the signal processing) may have affected the results.

The effect of IMF on backscatter parameters can be explained by its deposition inside the muscle perimysium. Muscular connective tissue is suggested to cause acoustic backscattering (Lizzi, Astor, Feleppa, Shao & Kalisz, 1997; Lizzi et al., 2003). While the perimysium is known to increase in thickness and mechanical strength with age, IMF is deposited in the perimysium during fattening of the muscle. Thus, disorganization and breakdown of the honeycomb structure results in a decrease of the mechanical strength of the muscle (Nishimura et al., 1999; Wood et al., 2008). A reduction in mechanical strength due to increased IMF (Nishimura et al., 1999; Wood et al., 2003) can therefore be considered to affect the ultrasonic backscatter intensity due to differences in size, structure and elastic properties, i.e. acoustic impedance of the connective tissue. Midband fit and apparent integrated backscatter are associated with the size and the acoustic impedance of the scattering structures (Lizzi et al., 1997; Lizzi et al., 2006).

Dependent on the axial resolution and frequency of the used ultrasound system, structural properties, e.g. scatter size and scatterer distance, are suggested to be explained by cepstral parameters. Therefore, an effect of the deposition of the IMF and the corresponding influence on structure and size of the connective tissue may partially be reflected by changes of the cepstral parameters. However, the spatial distance of the perimysial tissue is mainly affected by the size of the primary muscle bundles. With increasing bundle size, the distance between the layers of perimysium increases. Therefore, cepstral parameters are suggested to be mainly related to primary muscle bundle size and the effect of the IMF deposition inside the connective tissue is rather small. This is supported by the correlations found within the present study.

Minor positive relationships with the IMF have been found for backfat compound time of flight TOF_{BF}. This is in accordance with earlier investigations stating slightly positive relationships between backfat thickness and IMF (Moody and Zobrisky, 1966; Koch et al., 2010b). TOF_{BF} is mainly influenced by the the overall backfat thickness but also the proportion of skin (Koch et al., 2010b).

4.2 IMF prediction

The obtained linear correlations suggest the feasibility to predict the IMF content. Promising candidates were found among the spectral parameters. In the present investigation multiple linear regression yielded an R^2 of .76 and a RMSEP of 0.34 % IMF. A previous approach to predict porcine loin IMF with a large set of spectral parameters using partial least-squares regression (Mörlein et al., 2005) stated comparable results (R^2 = .58; RMSEP = 0.36 %). In contrast to spectral analysis, image analysis of in-vivo ultrasound scans allowed the prediction of porcine loin IMF with R^2 of .48 and an RMSEP of 0.71 % IMF (Maignel et al., 2010) and R^2 = .32; RMSEP: 1.02 %) (Newcom et al., 2002). B-mode image analysis of bovine *longissimus* muscle scans resulted in R^2 values comparable to the present investigation (up to 0.75) (Brethour, 1994; Hassen et al., 2001). Higher R^2 in beef may be explained by the broader IMF range (from 1 % to more than 11 %) of bovine muscle (Hassen et al., 2001).

Compared to the previous study (Mörlein et al., 2005), the number of acoustic parameters needed for IMF prediction could be reduced. This could be achieved by improved algorithms for ultrasound data pre-processing to correct for system specific and intermediate tissue effects. Furthermore, the ability to correctly classify 73 % of the carcasses into 3 IMF groups (thresholds at 1 and 2 %) proofs the practical use of ultrasound spectral analysis. The correct classification of 92 % of all carcasses into 2 classes is a further improvement to earlier investigations in which nearly 80 % of the carcasses were classified into 2 IMF groups (threshold at 2 % IMF) using discriminant analysis (Mörlein et al., 2005).

4.3 IMF variation

Longitudinal and cross-sectional variations of IMF have been stated in porcine muscle (Heylen, 1999) possibly compromising prediction accuracy. As for longitudinal variation, the IMF of adjacent loin chops 2 ribs apart differed by more than 1 % IMF (Heylen, 1999). Variations of more than 1 % IMF have also been stated for adjacent beef *longissimus* chops with a distance of 1 – 2 cm (Blumer, Craig, Pierce, Smart & Wise, 1962).

Besides longitudinal variations, significant differences of more than 0.7 % IMF were found between 5 cross-sectional regions of porcine loin chops with the highest IMF values for the ventral region (Heylen, 1999). In agreement to that, significant cross-sectional variation of IMF was observed in beef *longissimus* chops (Covington, Tuma, Grant & Dayton, 1970). As

the chemical IMF determination is performed at a homogenate of the complete chop it can be considered to be unaffected by cross-sectional inhomogenity. Contrarily, the ROI for ultrasound parameter estimation and subsequent IMF prediction is only a small subsample of the chop. As stated in table 1, the ROI size was 5.4 cm x 1.5 cm with a beam width of 1.2 mm. This results in a muscle volume of about 0.98 cm^3 covered by ultrasound. Furthermore, the ultrasound parameter estimation of the present investigation used a fixed region of interest within the -6 dB range of the transducer. Therefore, measurements performed at carcasses with a small backfat layer and a low attenuation may result in a ROI positioning near to the muscle / rib boundary while increasing backfat layers with higher attenuation would result in a shift of the focus position in dorsal direction, i.e. closer to the backfat where the IMF is lower compared to the ventral region (Heylen, 1999).

Therefore, the obtained RMSEP of 0.34 % IMF can be considered to be close to the possible optimum for predictions using such small regions of interest.

4.4 Excluded measurements

38 % of all measurements were discarded for several reasons given above, e.g. insufficient coupling or weak scattering. Within the present study, ultrasound parameter estimations were performed off line after careful inspection of the acquired data. For future applications, immediate data analysis after signal acquisition is needed to detect erroneous measurements and allow for replicate measurements if required. Several indicators for erroneous measurements were developed and applied, e.g. the number of excluded RF signals. Single gated RF signals close to the noise level (-40 dB) were excluded from further calculations. If the number of excluded gates exceeded 70 % of all gates within the ROI, the entire measurement was discarded.

Several data sets had to be excluded due to misplacement of the ROI, i.e. within the muscle-rib boundary. Thus, future adjustment of the acoustic lens or specific design of the transducer elements to shift the focus position to the centre of the muscle and to increase the -6 dB range are anticipated to improve the signal to noise ratio.

5. Conclusions and implications

Non-destructive IMF estimation via spectral analysis of the backscattered acoustic signal obtained on pig carcasses early post mortem (38 °C) has been described. Several algorithms for ultrasound data pre-processing were developed and applied before acoustic parameter estimation. Involved is an incorporation of recently determined reference parameters for backfat and muscle tissue, allowing to compute and correct for a potential shift of the sound field within the muscle tissue of interest.

Overall, multivariate regression analysis using a combination of three acoustic parameters, i.e. backfat time of flight, muscle attenuation, and spectral slope yields an average prediction error of 0.34 % IMF ($R^2 = 0.76$). MLR results enabled to correctly classify 92 % according to predicted IMF_{US} (threshold 2 % IMF). To further validate these results, the analysis of additional carcasses with the given calibration may be useful.

Predominant linear correlations to the IMF were found for attenuation, midband fit and spectral slope. The relationships with spectral parameters can be explained by the deposition of IMF inside the perimysium and corresponding alterations of its structure. Thus, acoustic backscattering is feasibly affected.

Compared to previous ultrasound based studies, considerably less acoustic parameters were needed to non-destructively predict the IMF of pork loin while the accuracy was slightly increased. Considering the longitudinal and cross-sectional variation of IMF and the system specific limitation of the ROI used for ultrasound data analysis, the achieved prediction error is suggested to be reasonable. Further improvements are anticipated by i) analysing multiple measurements at varying positions of the muscle, ii) adjustment of the acoustic lens to avoid ROI placement within the muscle / rib boundary, and iii) immediate data evaluation to detect erroneous measurements. Thus, further improvement of quantitative ultrasound devices for on line carcass and meat quality evaluation is feasible.

Acknowledgments - This work was supported by the German Research Council (DFG; grants Mo 1746/1 and Ra 1380/3), PRO INNO (grant KF0380401LF6) and Carometec GmbH. The authors thank U. Vehlow for the careful analysis of intramuscular fat content.

Reference List

Abou El Karam, S., Buquet, B., Berge, P. and Culioli, J. (1997). Ultrasonic characterization of bovine muscles. In *Proceedings of the 43rd ICoMST. 27 July to 1 August 1997. Auckland, New Zealand* (pp. 310-311).

Banihashemi, B., Vlad, R., Debeljevic, B., Giles, A., Kolios, M. C. and Czarnota, G. J. (2008). Ultrasound imaging of apoptosis in tumor response: novel preclinical monitoring of photodynamic therapy effects. *Cancer Res.*, 68, 8590-8596.

Benedito, J., Carcel, J. A., Rossello, C. and Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *MEAT SCIENCE*, 57, 365-370.

Bigelow, T. A. and O'Brien, W. D. (2005). A model for estimating ultrasound attenuation along the propagation path to the fetus from backscattered waveforms. *Journal Of The Acoustical Society Of America*, 118, 1210-1220.

Bigelow, T. A., Oelze, M. L. and O'Brien, W. D. (2005). Estimation of total attenuation and scatterer size from backscattered ultrasound waveforms. *Journal Of The Acoustical Society Of America*, 117, 1431-1439.

Blanchard, P. J., Warkup, C. C., Ellis, M., Willis, M. B. and Avery, P. (1999). The influence of the proportin of Duroc genes on growth, carcass and pork eating quality characteristics. *Animal Science*, 68, 495-501.

Blumer, T. N., Craig, H. B., Pierce, E. A., Smart, Jr. W. W. G. and Wise, M. B. (1962). Nature and variability of marbling deposits in *longissimus dorsi* muscle of beef carcasses. *J.Anim.Sci.*, 21, 935-942.

Brand, S., Mörlein, D. and Rosner, F. (2002). Estimation of intramuscular fat content (IMF) in the muscle longissimus dorsi (LD) of pigs by analysis of RF echo signals obtained from a clinical B-mode device. *IEEE Ultrason.Symp.*, 8-11.

Brethour, J. R. (1994). Estimating marbling score in live cattle from ultrasound images using pattern recognition and neural network procedures. *Journal of Animal Science*, 72, 1425-32.

Cantrell, J. H. Jr., Goans, R. E. and Roswell, R. L. (1978). Acoustic impedance variations at burn-nonburn interfaces in porcine skin. *J.Acoust.Soc.Am.*, 64, 731-735.

Covington, R. C., Tuma, H. J., Grant, D. L. and Dayton, A. D. (1970). Various Chemical and Histological Characteristics of Beef Muscle as Related to Tenderness. *J.Anim.Sci.*, 30, 191-196.

Essen-Gustavsson, B., Karlsson, A., Lundström, K. and Enfält, A. C. (1994). Intramuscular fat and muscle fibre lipid contents in Halothane-gene free pigs fed high or low protein diets and its relation to meat quality. *MEAT SCIENCE*, 38, 269-277.

Fernandez, X., Monin, G., Talmant, A., Mourot, J. and Lebret, B. (1999). Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of *M. longissimus lumborum*. *MEAT SCIENCE*, 53, 67-72.

Gammell, P. M., Croissette, D. H. L. and Heyser, R. C. (1979). Temperature and frequency dependence of ultrasonic attenuation in selected tissues. *Ultras.in Med & Biol*, 5, 269-277.

Gärtner, T. (2001). Ultraschallspektroskopie mit fokussierenden Systemen zur quantitativen Bestimmung von akustischen Parametern. In : Martin-Luther-Universität; Halle-Wittenberg.

Gelse, K., Olk, A., Eichhorn, S., Swoboda, B., Schoene, M. and Raum, K. (2010). Quantitative ultrasound biomicroscopy for the analysis of healthy and repair cartilage tissues. *Europ.Cells and Materials*, 19, 58-71.

Hassen, A., Wilson, D. E., Amin, V. R., Rouse, G. H. and Hays, C. L. (2001). Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *Journal of Animal Science*, 79, 11-18.

Hassen, A., Wilson, D. E. and Rouse, G. H. (2003). Estimation of genetic parameters for ultrasound-predicted percentage of intramuscular fat in Angus cattle using random regression models. *Journal of Animal Science*, 81, 35-45.

Heylen, K. (1999). Variation von anatomisch-physiologischen Merkmalen sowie Merkmalen der Fleischqualität im *M. longissimus thoracis et lumborum* des Schweins unter besonderer Berücksichtigung des intramuskulären Fettgehaltes. In .

Jensen, J. A. and Svendsen, N. B. (1992). Calculation of Pressure Fields from Arbitrarily Shaped, Apodized, and Excited Ultrasound Transducers. *IEEE Transactions On Ultrasonics Ferroelectrics And Frequency Control*, 39, 262-267.

Kipfmüller, H., Bodis, K., Peschke, W. and Eichinger, H. M. (2000). Qualität von Schweinefleisch. Beeinflussung durch das intramuskuläre Fett. *Ernährungsumschau*, 47, 416-422.

Koch, T., Brand, S., Sannachi, L., Raum, K., Wicke, M. and Mörlein, D. (2010a). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: I. Muscle. *MEAT SCIENCE*, accepted (DOI: 10.1016/j.meatsci.2010.12.002).

Koch, T., Brand, S., Sannachi, L., Raum, K., Wicke, M. and Mörlein, D. (2010b). Ultrasound velocity and attenuation of porcine soft tissues with respect to structure and composition: II. Skin and backfat. *MEAT SCIENCE*, accepted (DOI: 10.1016/j.meatsci.2010.12.004).

Lakshmanan, S., Koch, T., Mörlein, D., Brand, S. and Raum, K. (2010). Intramuscular fat content estimation in the loin muscle pig carcasses by ultrasound spectral parameter analysis. *IFMBE Proceedings*, 25, 92-95.

LFGB (2005). Bestimmung des Gesamtfettgehaltes in Fleisch und Fleischerzeugnissen. *Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB: L 06.00 6*.

Lizzi, F. L., Alam, S. K., Mikaelian, S., Lee, P. and Feleppa, E. J. (2006). On the statistics of ultrasonic spectral parameters. *Ultras.in Med & Biol*, 32, 1671-1685.

Lizzi, F. L., Astor, M., Feleppa, E. J., Shao, M. and Kalisz, A. (1997). Statistical framework for ultrasonic spectral parameter imaging. *Ultras.in Med & Biol*, 23, 1371-1382.

Lizzi, F. L., Astor, M., Liu, T., Deng, C., Coleman, D. J. and Silverman, R. H. (1997). Ultrasonic spectrum analysis for tissue assays and therapy evaluation. *Int.J.Imaging Syst.Technol.*, 8, 3-10.

Lizzi, F. L., Feleppa, E. J., Alam, S. K. and Deng, C. X. (2003). Ultrasonic spectrum analysis for tissue evaluation. *Pattern Recognition Letters*, 24, 637-658.

Madsen, E. L., et al. (1999). Interlaboratory comparison of ultrasonic backscatter, attenuation, and speed measurements. *J Ultrasound Med.*, 18, 615-631.

Madsen, E. L., Zagzebski, J. A., Banjavic, R. A. and Jutila, R. E. (1978). Tissue mimicking materials for ultrasound phantoms. *Med Phys*, 5, 391-394.

Maignel, L., Daigle, J. P., Gariépy, C., Wilson, D. and Sullivan, B. (2010). Prediction of intramuscular fat in live pigs using ultrasound technology and potential use in selection. *Proceedings of 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany (Nr.0668).*

Moody, W. G. and Zobrisky, S. E. (1966). Study of backfat layers of swine. *J.Anim.Sci.*, 25, 809-813.

Mörlein, D., Link, G., Werner, C. and Wicke, M. (2007). Suitability of three commercially produced pig breeds in Germany for a meat quality program with emphasis on drip loss and eating quality. *MEAT SCIENCE*, 77, 504-511.

Mörlein, D., Rosner, F., Brand, S., Jenderka, K. V. and Wicke, M. (2005). Non-destructive estimation of the intramuscular fat content of the *longissimus* muscle of pigs by means of spectral analysis of ultrasound echo signals. *MEAT SCIENCE*, 69, 187-199.

Newcom, D. W., Baas, T. J. and Lampe, J. F. (2002). Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *Journal of Animal Science*, 80, 3046-3052.

Ninoles, L., Clemente, G., Ventanas, S. and Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *MEAT SCIENCE*, 76, 102-111.

Nishimura, T., Hattori, A. and Takahashi, K. (1999). Structural Changes in Intramuscular Connective Tissue During the Fattening of Japanese Black Cattle: Effect of Marbling on Beef Tenderization. *Journal of Animal Science*, 77, 93-104.

Oppenheim, A. V. and Schafer, R. W. (2004). From Frequency to Quefrency: A History of the Cepstrum. *IEEE Signal Processing Magazine*, 21, 95-106.

Park, B., Whittaker, A. D., Miller, R. K. and Bray, D. E. (1994). Measuring Intramsucular Fat in Beef with Ultrasonic Frequency Analysis. *Journal of Animal Science*, 72, 117-125.

Park, B., Whittaker, A. D., Miller, R. K. and Hale, D. S. (1994). Predicting Intramuscular Fat in Beef Longissimus Muscle from Speed of Sound. *Journal of Animal Science*, 72, 109-116.

Raju, B. I. and Srinivasan, M. A. (2001). High-frequency ultrasonic attenuation and backscatter coefficients of in vivo normal human dermis and subcutaneous fat. *Ultrasound in Med.& Biol.*, 27, 1543-1556.

Raum, K. (2008). Microelastic imaging of bone. *IEEE Transactions On Ultrasonics Ferroelectrics And Frequency Control*, 55, 1417-1431.

Raum, K., Kempf, K., Hein, H. J., Schubert, J. and Maurer, P. (2007). Preservation of microelastic properties of dentin and tooth enamel in vitro--a scanning acoustic microscopy study. *Dent.Mater.*, 23, 1221-1228.

Raum, K. and O'Brien, W. D. Jr. (1997). Pulse-Echo Field Distribution Measurements Technique for High-Frequency Ultrasound Sources. *IEEE Trans Ultrason Ferroelectr Freq Control.*, 44, 810-815.

Raum, K., Reisshauer, J. and Brandt, J. (2004). Frequency and resolution dependence of the anisotropic impedance estimation in cortical bone using time-resolved scanning acoustic microscopy. *Journal of Biomedical Materials Research Part A*, 71A, 430-438.

Scheipers, U., Ermert, H., Sommerfeld, H. J., Garcia-Schürmann, M., Senge, T. and Philippou, S. (2003). Ultrasonic multifeature tissue characterization for prostata diagnostics. *Ultras.in Med & Biol*, 29, 1137-1149.

Smith, N. B. (1996). Effect of myofibril length and tissue constituents on acoustic propagation properties of muscle. *Ph.D.Thesis, University of Illinois.*

Suzuki, K., Irie, M., Kadowaki, H., Shibata, T., Kumagai, M. and Nishida, A. (2005). Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. *J.Anim.Sci.*, 83, 2058-2065.

Ville, H., et al. (1997). An evaluation of ultrasound and nuclear magnetic resonance spectroscopy to measure in vivo intramuscular fat content of longissimus muscle of pigs. *J.Anim.Sci.*, 75, 2942-2949.

Whittaker, A. D., Park, B., Thane, B. R., Miller, R. K. and Savell, J. W. (1992). Principles of Ultrasound and Measurement of Intramuscular Fat. *J.Anim.Sci.*, 70, 942-952.

Wood, J. D., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *MEAT SCIENCE*, 78, 343-358.

Wood, J. D., et al. (2003). Effects of fatty acids on meat quality: a review. *MEAT SCIENCE*, 66, 21-32.

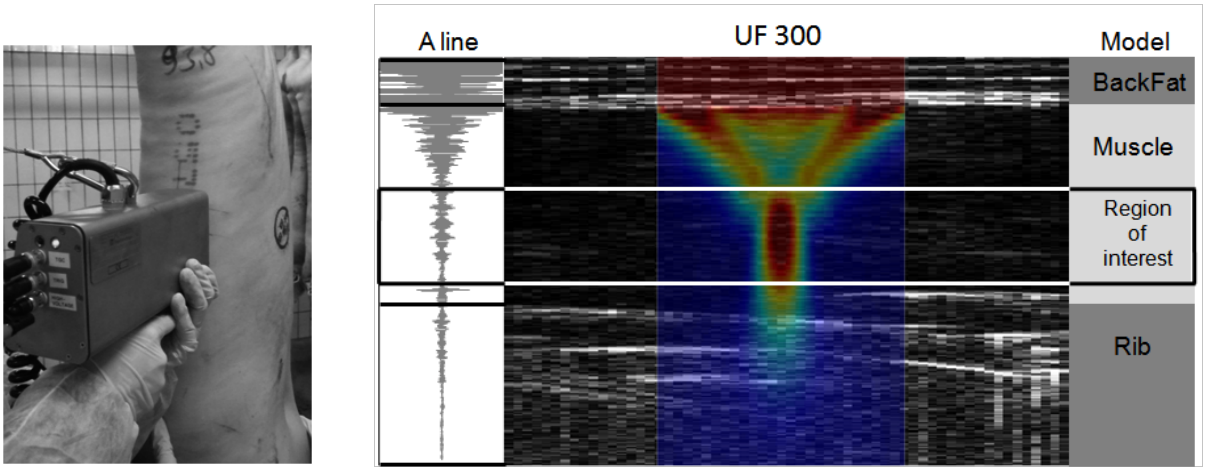


Figure 1: Ultrasound data acquisition on hanging pig carcasses at slaughter (left). Gray scale B-mode image of porcine muscle and backfat, overlaid color coded acoustic focus and the corresponding RF signal (A line) are illustrated (right).

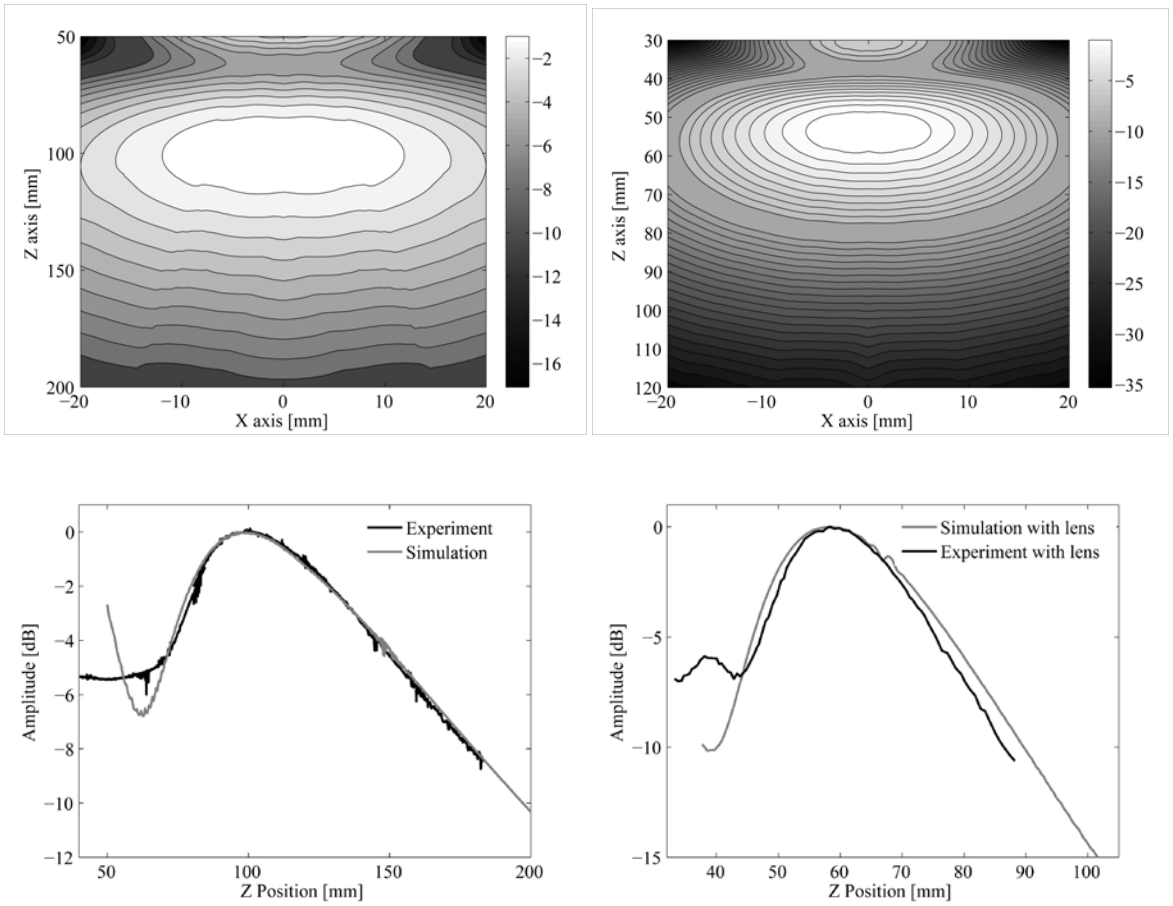


Figure 2: Contour plot of spatial intensity distribution in dB of UF300 without (upper left) and with (upper right) acoustic lens. Sound field plots along the depth estimated from simulation and experiment without (lower left) and with acoustic lens (lower right).

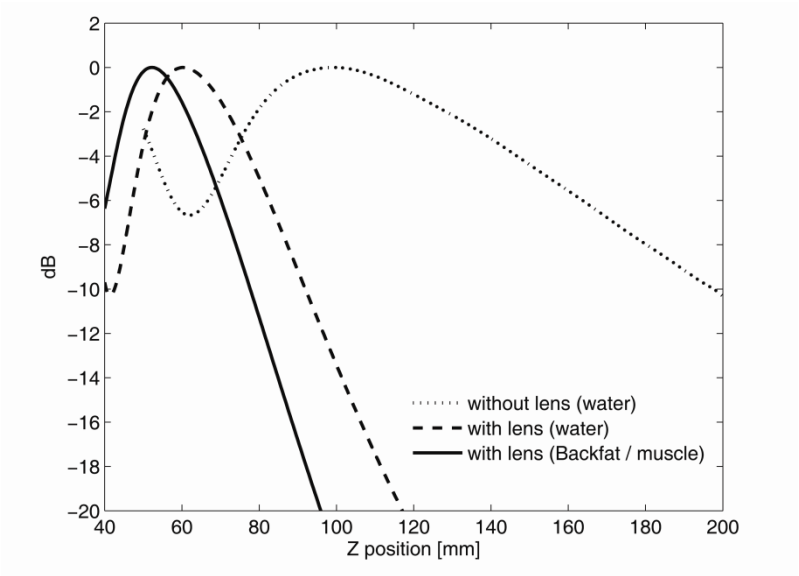


Figure 3: Sound field plots along the depth with and without acoustic lens

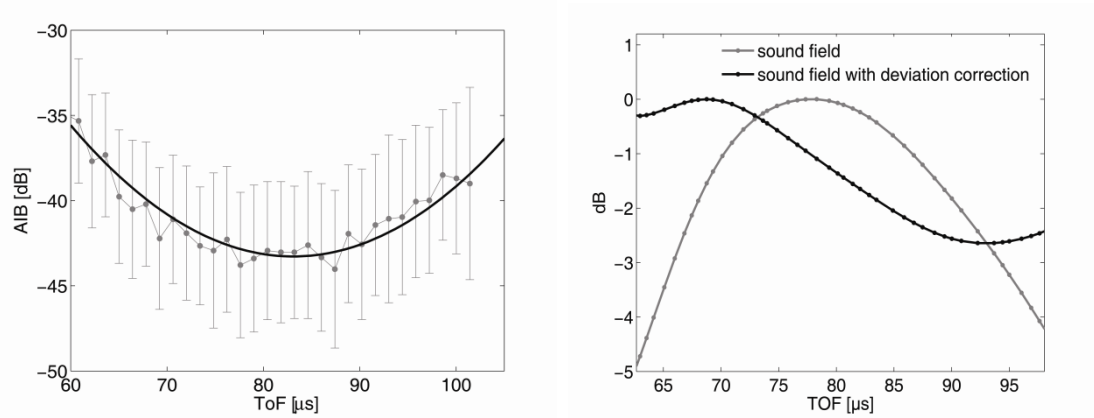


Figure 4: AIB estimation before WFCC correction (left). Axial intensity distribution and WFCC-corrected AIB (right). For better illustration, a bias of 40 dB was added.

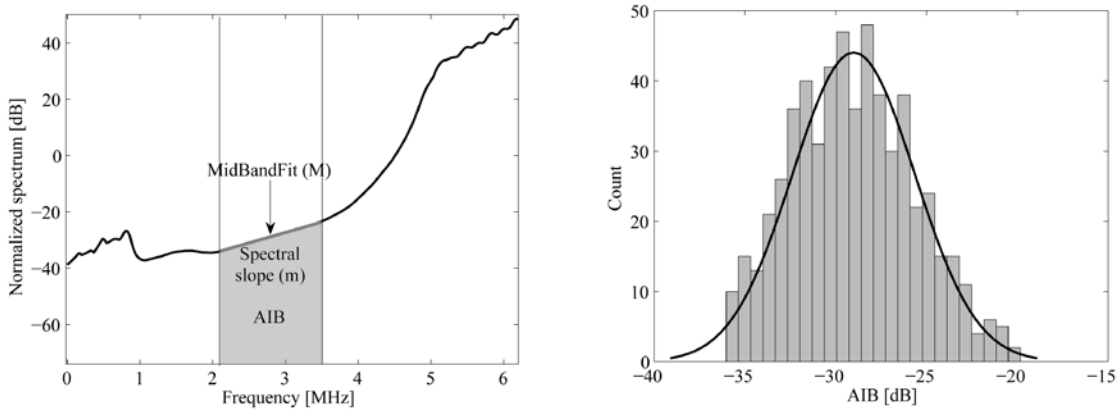


Figure 5: Estimation of spectral parameters from the normalized power spectrum (left). AIB histogram distribution within the evaluated muscle region of interest (right).

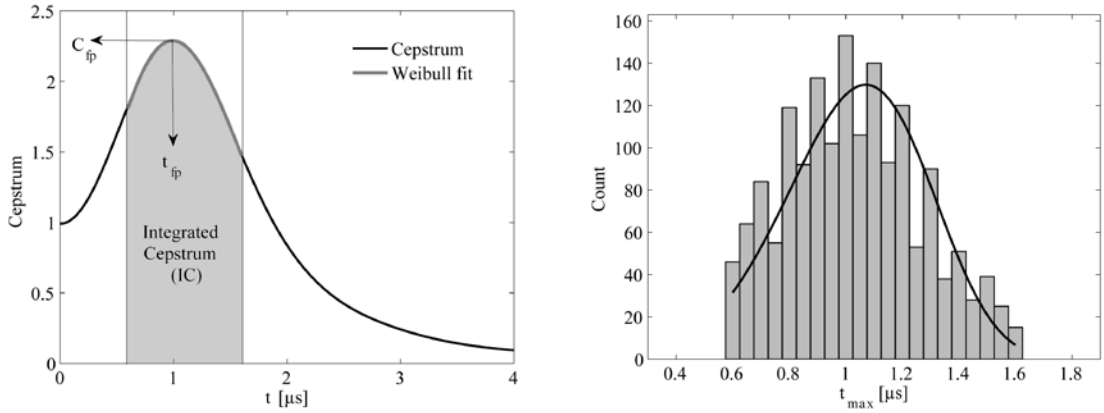


Figure 6: Cepstral parameters estimation (left). Cepstral parameter, τ_p distribution within region of interest with weibull fit (right).

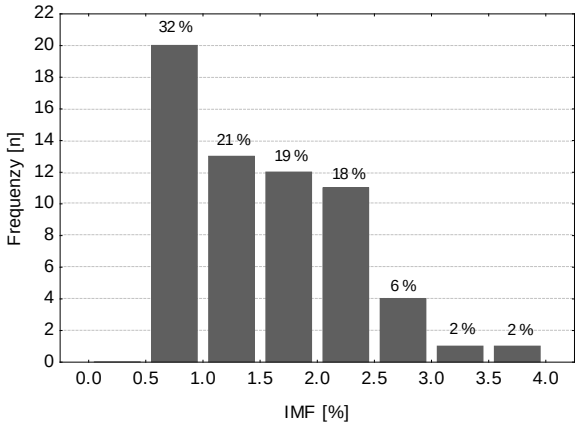


Figure 7: Distribution of the chemically determined IMF in porcine *longissimus* muscle (n = 62).

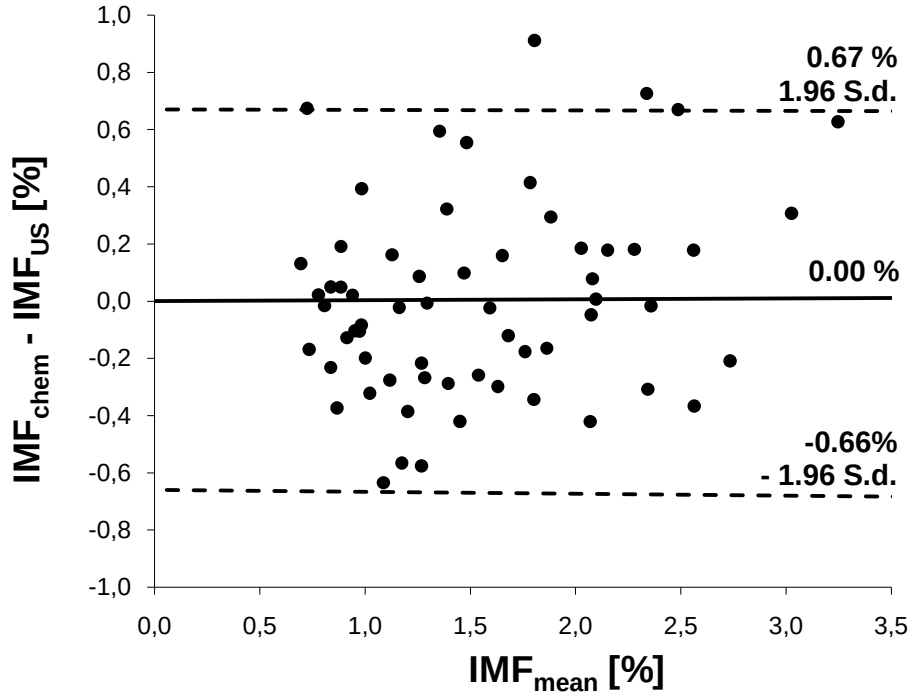


Figure 8: Bland-Altman plot of chemically and via ultrasound determined IMF (n = 62). Given is the mean value of both methods and the ± 1.96 * standard deviation.

Table 1: Physical parameters and sound field characteristics of Ultrafom 300 measured in water at 38 °C.

Physical Parameters		Beam Parameters		
Type	Linear Array	UF300	*Without lens*	*With lens*
No. of elements	64	Pulse duration ($\tau_{(-20\ dB)}$)	0.96 µs	1.5 µs
Pitch	0.80 mm	Center frequency	3.2 MHz	2.7 MHz
Kerf	0.051 mm	Frequency bandwidth	2.4 – 4.0 MHz	2.1 – 3.5 MHz
		Range f_1 - f_2 (-6 dB)		
Elevation	18 mm	Focus position	95.8 mm	60.8 mm
Elevation ROC	100 mm	Depth of focus (-6 dB)	96.5 mm	35.2 mm
		Elevation Beam width,	2.5 mm	1.2 mm
		ΔY (-6 dB)		
		Lateral Beam width,	53.6 mm	28.8 mm
		ΔX (-6 dB)		

Table 2: Acoustic properties of agar phantoms and different tissues.

	Sound velocity [ms^{-1}]	Attenuation [dB MHz^{-1} cm^{-1}]	Source	Notes
Agar	1550	0.2 – 0.3	(Madsen et al., 1978)	Adjustable from 0.2 to 1.5 dB MHz^{-1} cm^{-1} and 1520 to 1650 ms^{-1}.
Agar/graphite	1540 ± 15	0.5 ± 0.05	(Madsen et al., 1978)	
Muscle	1621 ± 5	1.02 ± 0.26	(Koch et al., 2010a)	
Skin	1682 ± 23	2.5 ± 0.6	(Koch et al., 2010b)	
Skin	1710 ± 60	0.7	(Cantrell et al., 1978)	Measured at 25° C
Fat	1450 ± 27	1.89 ± 1.05	(Koch et al., 2010b)	Different between the fat layers
Fat	1530		(Ninoles et al., 2007)	Measured at 20° C
Backfat compound	1502 ± 21	1.59 – 2.27	(Koch et al., 2010b)	
Backfat compound		2.0 – 2.5	(Gammell et al., 1979)	Only one animal

Table 3: Acoustic spectral and cepstral parameters extracted from ultrasound rf data

Parameters	Units	Description
TOF_{BF}	µs	Backfat compound time of flight
BW	mm	Backfat compound thickness
SOS_{BF}	m/s	Backfat compound speed of sound
α_{muscle}	dB/MHz/cm	Muscle attenuation coefficient
AIB	dB	Mean of apparent integrated backscatter amplitude
M	dB	MidBandfit
m	dB/MHz	Backscatter power spectral slope
RF_{excl}	%	Percent of rf signals correspond to spectral intensity below -40 dB excluded
IC	dB	Integrated cepstrum
$C_{fp}(\tau_{fp})$	dB	Cepstrum first peak amplitude
τ_{fp}	µs	Cepstrum first peak amplitude position

Table 4: Mean values, standard deviation, coefficient of variation CV, minimum and maximum of all evaluated carcass parameters.

	n	Mean ± STD	CV [%]	Minimum	Maximum
Intramuscular fat [%]	62	1.53 ± 0.69	44.8	0.65	3.56
Carcass weight [kg]	62	95.9 ± 6.4	6.7	80.0	112.0
Lean meat percentage [%]	62	54.3 ± 3.6	6.6	47.7	61.0
Muscle width [mm]	61	60.5 ± 6.0	9.9	47.1	74.5
Backfat width [mm]	61	18.9 ± 3.8	20.2	11.8	26.4
TOF_{BF} [µs]	62	20.1 ± 4.5	22.2	11.9	30.4
α_{muscle} [dB $MHz^{-1}cm^{-1}$]	62	0.77 ± 0.15	19.3	0.54	1.17
AIB [dB]	62	-34.1 ± 4.6	13.5	-42.7	-22.3
M [dB]	62	-34.0 ± 4.4	13.0	-42.1	-22.8
m [dB MHz^{-1}]	62	4.07 ± 1.17	28.77	1.25	6.33
Excluded RF-signals [%]	62	24.5 ± 14.6	59.6	1.4	61.1
IC [dB]	62	2.01 ± 0.10	5.02	1.78	2.23
$C_{fp}(\tau_{fp})$ [dB]	62	2.33 ± 0.14	5.81	2.07	2.64
τ_{fp} [µs]	62	1.02 ± 0.01	1.33	0.99	1.05

Table 5: Linear correlation coefficient (r) of acoustic parameters with intramuscular fat in porcine *longissimus* muscle.

TOF_{BF}	**.27**	*	RF_{excl}	-.17	
SOS_{BF}	-.25		IC	**.26**	*
α_{muscle}	**.61**	**	$C_{fp}(\tau_{fp})$	**.29**	*
AIB	**.56**	**	τ_{fp}	.10	
M	**.56**	**	τ_{fp} (s.d.)	-.21	
m	.04				

Significant correlations are marked with: * < .05; ** < .001

Table 6: Classification results of 3 classes of chemically determined IMF (IMF_{chem}) measured on the porcine *longissimus* muscle and based on predicted fat content (IMF_{US}). The thresholds were chosen: < 1 % IMF (LOW), 1 to 2 % IMF (MID) and > 2 % IMF (HIGH); (n=62)

	IMF_{chem} LOW	IMF_{chem} MID	IMF_{chem} HIGH
IMF_{US} LOW	**10 (50 %)**	2 (8 %)	
IMF_{US} MID	10 (50 %)	**22 (88 %)**	4 (24 %)
IMF_{US} HIGH		1 (4 %)	**13 (76 %)**

Overall error rate: 27 %

5 Discussion

The following chapter is intended to summarize the results obtained during the present investigations and to target some of the aspects not discussed by now. This includes the influence of the reference data collected with scanning acoustic microscopy on hot carcasses measurements. Furthermore, procedural problems of the presented ultrasound systems under slaughterhouse conditions will be discussed. Finally, an outlook will be given on future developments and gaps in knowledge which should be targeted in further investigations.

5.1 Impact of scanning acoustic microscope results

The major topic of the measurements performed with a scanning acoustic microscope was to collect reference data for muscle and backfat samples comparable to hot carcass conditions. Both investigations revealed values slightly differing from earlier results (Chivers and Parry, 1978; Greenleaf, 1986) and reference values actually used in most ultrasound devices (e.g. 1540 ms^{-1} for all tissue types in medical ultrasound devices). Furthermore, relationships have been stated between compositional and / or structural traits and the investigated ultrasound parameters. The amount of intramuscular fat has been shown to be the most important parameter influencing attenuation and, to a lesser extent, sound velocity of the muscle. Contrary, even if the amount and distribution of fatty acids influences the sound velocity of individual backfat layers, the velocity of the compound sample is mainly determined by the skin / fat ratio and therefore the overall backfat width. This is one of the major findings of the present investigation as it allows a thickness dependent correction for the sound velocity of backfat layers.

The obtained reference values were thereafter included into the correction algorithms of a hand-held ultrasound device (UltraFOM300; Carometec; Denmark). These new reference values were suggested to improve the sound field correction and therewith also the accuracy of IMF prediction. Moreover, they can be expected to improve muscle and backfat thickness estimation via ultrasound, requiring an accurate sound velocity of the investigated tissue. The following chapters will give an overview of the impact the obtained reference values had.

5.1.1 Influence on backfat thickness estimation

A more common usage of ultrasound in meat industry involves backfat and loin muscle thickness estimation and the corresponding calculation of lean meat percentage. Most of the used systems are either hand held devices (UltraFOM 300, Carometec, Denmark) or fully automated carcass classification systems (AutoFOM, Carometec, Denmark) (Broendum, Egebo, Agerskov & Busk, 1998; Fortin, Tong & Robertson, 2004). As the payment system for pig producers is based on the lean meat percentage, the accuracy of these systems is commercially important. However, during the last years a difference between tissue thickness obtained via ultrasound and inversion probe or ruler has been repeatedly reported for a wide range of different animals (Brethour, 1992; Greiner, Rouse, Wilson, Cundiff & Wheeler, 2003; Ripoll, Joy, Alvarez-Rodriguez, Sanz & Teixeira, 2010). Mostly those deviations have been referred to user effects, muscle contraction or post mortem carcass handling. Apparently, one source of error has rarely been discussed by now.

Ultrasound thickness measurements are based on the time of flight of the acoustic wave inside the tissue and calculated by the relation of those TOF to pre-set sound velocity values. Sound velocity values however, are highly differing between tissue types (e.g. fat, muscle, skin) as shown in the present study. Thus, high attention should be given to select accurate values for a given tissue. Unfortunately, a wide range of measurements are still performed with inadequate settings and are thus object of inaccuracies. Most modern carcass classification systems (e.g. AutoFOM) use different values for backfat (e.g. 1440 ms^{-1}) and muscle (e.g. 1580 ms^{-1}). However, as shown earlier the sound velocity values of skin and fat differ significantly. Therefore, a more accurate estimation of backfat thickness could be expected using differing values for these tissue types. To predict this potential influence, the thickness estimations of a fixed and two different sound velocity values for skin and fat have been compared with each other theoretically. All sound velocity values were chosen in accordance to the mean values obtained via scanning acoustic microscopy measurements. The theoretical thickness values for skin and backfat were selected in a range comparable to the carcasses investigated during the present study. All calculations have been performed using equation 1. Due to the fact that a reflected signal is analysed, the acoustic wave has to pass every tissue twice which has to be considered in the equation:

$$Velocity\ [m\ s^{-1}] = 2x(Thickness\ [mm]\ Time\ of\ Flight^{-1}\ [\mu s^{-1}]) \quad (1)$$

Firstly, differing sound velocities for fat (1450 m s^{-1}) and skin (1680 m s^{-1}) have been used to calculate the time of flight of the acoustic wave inside the individual layers with thickness values from 2 to 4 mm (skin) and 5 to 20 mm (fat) (equations 2 and 3):

$$Time\ of\ Flight_{Fat}[\mu s] = 2x(Thickness\ [mm]\ x\ 1.45\ [\mu s\ mm^{-1}]) \quad (2)$$

$$Time\ of\ Flight_{Skin}[\mu s] = 2x(Thickness\ [mm]\ x\ 1.68\ [\mu s\ mm^{-1}]) \quad (3)$$

The obtained TOF_{fat} and TOF_{skin} values have been added to calculate the $TOF_{backfat}$ of the backfat compound sample. Thereafter, $TOF_{backfat}$ was used to calculate the thickness of the backfat if only one fixed sound velocity (1499 ms^{-1}) is used (equation 4):

$$Thickness_{1499}[mm] = (Time\ of\ Flight_{Fat}[\mu s]\ x\ 1.499\ [\mu s\ mm^{-1}]) / 2 \quad (4)$$

The obtained calculated backfat thickness has been compared to the preassigned thickness of the complete backfat.

As can be seen in table 1, a constant sound velocity leads to a prediction error in relation to the percentage amount of the skin compared to the fat. Smaller samples with a higher amount of skin are more subject to thickness underestimation, (up to -0.3 mm) while thick fat layers with low amounts of skin are overestimated (up to 0.5 mm).

The obtained results are in accordance to earlier investigations measuring the backfat thickness of cattle and pig using ultrasound (Brethour, 1992; Moeller and Christian, 1998). As in the present investigation, thicker backfat layers have been described to be more subject to overestimation than small backfat layers. This can be explained by the higher sound velocity of the skin. A high percentage amount of skin (small fat, thick skin) results in a lower TOF for a given thickness. However, using a constant sound velocity will not differentiate between skin and fat and the reduced TOF is therefore associated with a lower backfat thickness.

Table 1: Estimation of porcine backfat thickness using individual sound velocity values for fat (1450 ms^{-1}) and skin (1680 ms^{-1}) compared to an average sound velocity of 1499 ms^{-1}.

Skin [mm]	Fat [mm]	Block [mm]	TOF skin [µs]	TOF fat [µs]	TOF backfat [µs]	Thick 1499 [mm]	Dif. 1499 [mm]	Dif. 1499 [%]
2	5	7	2.38	6.90	9.28	6.96	**-0.04**	**-0.57**
2	10	12	2.36	13.79	16.17	12.12	**0.12**	**1.00**
2	15	17	2.38	20.69	23.07	17.29	**0.29**	**1.71**
2	20	22	2.38	27.59	29.97	22.46	**0.46**	**2.09**
3	5	8	3.57	6.90	10.47	7.85	**-0.15**	**-1.88**
3	10	13	3.57	13.79	17.36	13.01	**0.01**	**0.08**
3	15	18	3.57	20.69	24.26	18.18	**0.18**	**1.00**
3	20	23	3.57	27.59	31.16	23.35	**0.35**	**1.52**
4	5	9	4.76	6.90	11.66	8.74	**-0.26**	**-2.89**
4	10	14	4.76	13.79	18.55	13.90	**-0.10**	**-0.71**
4	15	19	4.76	20.69	25.45	19.07	**0.07**	**0.37**
4	20	24	4.76	27.59	32.35	24.25	**0.25**	**1.04**

TOF: time of flight; Thick: calculated thickness with backfat sound velocity of 1499 ms^{-1}; Dif: difference between thickness calculation with 1499 ms^{-1} and differing values for skin and fat

According to these results, a fixed sound velocity value for thickness prediction will in most cases lead to a wrong estimation of the real backfat thickness. Therefore, the usage of individual sound velocity values for skin and fat should improve backfat thickness estimation. This will also improve the prediction of lean meat percentage, especially for backfat with either a considerably high or low percentage amount of skin.

To evaluate this theoretical approach, different methods of backfat thickness calculation have been investigated on 62 animals measured with a hand-held ultrasound device (UltraFom300; Carometec; Denmark). As shown in table 1, using a fixed sound velocity will almost always lead to differences between the real and estimated backfat thickness in relationship to the overall skin percentage. In theory, this lack in accuracy can be overcome using two different sound velocity values for skin and fat. Therefore, a fixed sound velocity (1499 ms^{-1}) and differing sound velocities for skin (1680 ms^{-1}) and fat (1450 ms^{-1}) have been implemented in the backfat correction algorithms used for the UltraFom300 systems with otherwise constant settings.

As a result, the obtained mean deviations of all backfat thickness values differed by less than 0.2 mm between both correction methods. This only slight (and not significant)

improvement may be reasoned by the fact that the amount of samples with either high or low skin percentages was rather low. Out of the 44 samples being measured with a ruler the majority (about 3/4) showed a mediocre skin percentage (between 15 and 25 %) being only slightly affected by differing sound velocity settings (as can be seen in table 1).

It should be noted however, that the results of both backfat correction methods showed significantly lower (about 3.8 mm) thickness values compared to the ones obtained via FOM. This is in accordance to earlier publications. Ripoll et al. (2010) stated up to 2 mm smaller backfat layers measured with ultrasound at living lambs compared to carcass measurements with a ruler. Comparable findings have been stated by Moeller and Christian (1998) mentioning backfat thickness deviations of about 1.15 mm between ultrasound measurements and standardised carcass collection procedures at pigs. Again, the smaller values were found for the ultrasound measurements.

A possible reason may be the fact that FOM measurements were performed at individual positions of the carcass, while ultrasound measurements were carried out over a length of about 6 cm in the present investigation. Measurements via ruler and image analysis performed during the present investigation at excised chops at 3 positions along the backfat also revealed thickness values about 2 mm lower than the FOM measurements. Furthermore, a slight pressure on the carcass during the measurement to enable coupling may also reduce the effective thickness partially explaining the remaining variance.

5.1.2 Backfat correction

As stated above, more accurate sound velocity values for skin and fat layers do not significantly affect thickness estimation of the backfat layers. However, improvement of backfat thickness estimation was never the target of the present investigation. The new sound velocity and attenuation values were implemented into the correction algorithms of an ultrasound device required for the exact form and positioning of the sound field. Overall, an improvement in IMF prediction could be obtained compared to earlier investigations analyzing unprocessed backscatter data (Mörlein, et al, 2005) using fewer predictive variables. However, to enable the most accurate sound field correction, different methods can be used calculating the sound velocity and the thickness of the backfat layer out of the measured TOF values. This includes the above mentioned use of a constant sound velocity and the use of different values for skin and fat but also an iterative minimization technique.

To evaluate the influence of the obtained reference values and the used backfat correction calculations, three different methods have been applied, compared with each other and related to the chemically determined IMF:

I. Constant backfat and sound velocity values have been assumed as a minimum correction. Both values were chosen in accordance to the via SAM measurements obtained mean values (SOS: 1499 ms^{-1}; backfat thickness: 14.54 mm).

II. Different SOS values were chosen for the skin (1450 ms^{-1}) and the combined fat layers (1680 ms^{-1}) also in accordance to the SAM measurements.

III. An iterative minimization algorithm was applied to predict backfat SOS and thickness out of the corresponding TOF value (Lakshmanan, Koch, Mörlein, Brand & Raum, 2009). Briefly, a constant sound velocity was assigned to predict the backfat in a first step. The obtained thickness was used to calculate a new, thickness corrected sound velocity. Thereafter, new thickness and sound velocity values were obtained repeating this procedure until the difference between two consecutive calculation steps was less than 0.1 ms^{-1}.

Comparing the obtained mean values and standard deviations of all 3 backfat correction settings, an analysis of variance revealed no significant differences for any of the investigated US parameters. In accordance to this, linear correlations between acoustic parameters and IMF showed only minor variations (not significant) between the investigated backfat correction methods. MLR was applied using automatic detection of the best combination of ultrasound parameters to predict the IMF. Best results could be obtained for the iteration method (III) as used in the attached publication (R^2 = .76; RMSEP: 0.34 % IMF). However, the differences between all 3 methods were minor and not significant (R^2 from .74 to .76) using the same number of predictive variables (3). The predictive variables attenuation and spectral slope were implemented in the model independent from the correction method. Contrary, the time of flight inside the backfat was only used for the correction methods II and III while apparent integrated backscatter was implemented for method I.

Overall, the differences between the investigated backfat correction methods on spectral parameters are only minor ones. This can partially be explained by the small variation in the obtained speed of sound values of the backfat layers between the correction methods.

The mean values of all 3 methods were within 18 ms^{-1} from each other. Considering these results, even if an adequate correction function is required for the exact placement and form of the sound field, only minor differences can be stated between the different backfat correction methods investigated during this study.

5.2 Evaluation of procedural problems and potential errors

As described in the present publications a range of pigs with IMF levels and lean meat percentages representative for German pig populations has been analyzed. However, even if this selection worked for the mentioned parameters, there are still limitations to a rather small range of additional factors like age and genetic. Both parameters however, are known to affect structural and compositional traits of backfat and muscle. Older animals were mentioned to have increased amounts of connective tissue together with an increased muscle fibre and bundle diameter (Fang, Nishimura & Takahashi, 1999; Lefaucheur, 2010). More cross-links in the connective tissue of the muscle have been stated to harden the muscle (Ahhmed, Nasu, Huy, Tomisaka, Kawahara & Muguruma, 2009) and may also affect attenuation and backscatter properties. Variations in the amount of IMF have been stated both cross-sectional and longitudinal the muscle. This may affect measurements performed at considerably small areas of the muscle. Furthermore, the percentage amount of the inner fat layer and the overall backfat thickness is larger in older animals (Moody and Zobrisky, 1966) while thickness and structure of the skin differ between breeds (Meyer, Neurand & Radke, 1982). Those factors and their potential influence on ultrasound measurements will be discussed in the following.

5.2.1 Variations between animals

With increasing age of an animal the structure and composition of both muscle and backfat varies. While the amount of muscle fibres is constant after birth, their size increases during growth (Zgur, 1991). In addition, size and amount of cross-links inside the connective tissue increase with age being associated with an increased stability and shear-force value (Fang et al., 1999). The amount and constitution of collagen is considered to be one of the parameters responsible for the backscatter of the ultrasound signal (Lizzi, Feleppa, Alam & Deng, 2003). Lizzi (1997) has introduced several spectral parameters (including midband fit and spectral slope) that are related to the structure of the scatterers. Therefore, an influence of

aging on ultrasound backscatter analysis could be expected. These findings have already been proven for medical diagnostics on human muscle showing significantly higher backscatter levels measured at the experimental group aged 41 years and older compared to the younger (≤ 40 years) group (Zaidman, Holland, Anderson & Pestronk, 2008). Besides age, the amount of collagen and shear-force values also differs between pig breeds (Baland and Monin, 1987; Brewer, Jensen, Sosnicki, Fields, Wilson & McKeith, 2002). Considering these results, adjustments of the correction algorithms may be required for measurements on animals of different ages.

During the present investigations the percentage amount of skin (ranging from less than 10 % to more than 25 %) was found to be highly important for the compound backfat sound velocity. This is reasoned in high differences between the sound velocities of skin and fat. However, while the sound velocity of the skin showed a mediocre variation (1682 ± 23 ms^{-1}) genetic or procedural differences my alter those values and therefore the described relationships. Out of the procedural differences mainly scalding time and temperature have to be mentioned. Even if the overall procedure is comparable in most German slaughterhouses, minor differences can't be avoided. A significant increase in destruction of the outer skin regions has been stated with increasing scalding time and temperature (Mowafy and Cassens, 1975; Cantrell, Goans & Roswell, 1978) which may potentially alter ultrasound characteristics. Even if Cantrell (1978) could not state any significant influence on sound velocity analyzing the skin of 2 pigs, increasing the burning time from 20 to 60 seconds (100 °C) increased the attenuation of the skin by about 70%.

In addition, significant structural and compositional differences of the skin between different breeds have to be considered (Meyer et al., 1982). Attenuation differences have been mentioned for skin obtained from different carcass positions (Baldeweck, Laugier & Berger, 1995).

5.2.2 IMF variation within the muscle

For both scientific studies and determination of the carcass value the position of the 2^{nd} / 3^{rd} last rib of the *longissimus* muscle is widely considered as reference value mainly due to the comparably high homogeneity of the IMF at this position (Heylen, 1999). However,

there are still differences in the IMF content both longitudinal and cross-sectional the muscle. Significantly higher amounts of IMF have been stated in both *caudal* and *cranial* directions of the muscle with lowest values at the 2nd / 3rd last rib measuring the IMF of one chop every second rib (Heylen, 1999). Variations in the range of more than 1 % IMF have also been stated for beef chops divided only 1 to 2 cm from each other analyzing both, ether extracted amount of fat and marbling score (Blumer, Craig, Pierce, Smart & Wise, 1962). Furthermore, significant differences of more than 0.7 % IMF have been mentioned at different cross-sectional positions for both bovine and porcine *longissimus* muscle (Covington, Tuma, Grant & Dayton, 1970; Heylen, 1999).

As the IMF determination via ether extraction during the present investigation used a homogenate of a whole chop of sufficient thickness (about 2 cm), the results can be considered to be mostly independent from the above stated cross-sectional variation. Taking the high IMF variations into account however, the region of interest used for ultrasound measurements may appear too small to be comparably representative. For investigations with the SAM the sample size never exceeded 1 cm^3. As stated above, even an adjacent sample may have slightly differing IMF contents. This is even more feasible considering the results obtained for both native samples via SAM measurements and the differences in correlations between US and compositional parameters. The same problem can be found using ultrasound systems to predict the IMF on carcasses. While a region of interest of 5.4 cm along the muscle and a depth of 1.5 cm sounds considerably large, in sum this result in an overall volume of about 0.97 cm^3 as the beam width is only 0.12 cm. As already stated, considerable variations in the IMF content could therefore be expected changing the measurement position. Thus, methods predicting the IMF require a representative amount of muscle tissue to avoid regional differences and to improve the results. Options to provide such a representative sample size depend on the used system. Samples used for SAM measurements are highly limited in size (due to the setup) and thickness (due to the high frequency used). Therefore, multiple samples should be measured to improve reliability. Measurements with a diagnostic ultrasound device are comparably quick and easy to perform. The use of multiple measurements with slightly different positions and / or orientations could help to improve the results. During the present investigations, up to 3 measurements were performed per carcass. However, all measurements were performed at the same position with comparable orientations of the transducer. Nonetheless, even the mean values out of those measurements revealed a significant improvement of the IMF prediction (as shown in 5.2.4). However, a further increase may be achieved measuring multiple positions longitudinal and / or cross-sectional the muscle.

5.2.3 Influence of handling during measurements on hanging carcasses

Even if all measurements of the present investigation were performed by experienced users, small differences in handling (e.g. pressure or angle) are hard to avoid and may have affected the results. Smith (1996) mentioned attenuation variations up to 0.1 dB MHz^{-1} cm^{-1} changing the angle of insonification in relation to the muscle fibre orientation by 5° measured on shear muscle samples. Concurrent, sound velocity differed by less than 2 ms^{-1}. Even if these measurements were performed under laboratory conditions on shear muscle samples, an influence of the angle on hot carcass measurements may still be plausible and could affect IMF prediction. A template for correct placement of the transducer was not used during the present investigation but is already in use for modern systems and ensures a correct angle of insonification. Nonetheless, additional measurements should be conducted on hot carcasses to evaluate the potential influence of differing angles on the obtained acoustic parameters.

Besides an imprecise angle, the pressure used to connect the transducer with the carcass may also influence the results. As already stated, about 10 % of the measurements performed during the present investigation with the UltraFom300 ultrasound device had to be deleted due to insufficient coupling. One reason may be the rounded shape of the carcass complicating a complete attachment of the whole transducer. To prevent inadequate coupling, an increase of the contact pressure is required to ensure a complete connection between the lens and the carcass. This increased pressure however, may affect the measurements mainly due to a compression of the tissue (backfat and muscle) altering its thickness and potentially also acoustic parameters. Hardware or software adaptations (as discussed later on) detecting either an increased contact pressure or measurements with insufficient coupling could help to avoid those problems.

5.2.4 Repeatability and multiple measurements

A major factor important for the approval and application of newly developed systems are consistent results for repeated measurements. To target this so called intraclass correlation or repeatability, mean values are calculated out of the squared deviations of measurements on different animals (variability) and consecutive measurements on one animal (residual) using equation 5 (Bland and Altman, 1999). The obtained results are given as values between r = 0.0 (no repeatability) and r = 1.0 (perfect repeatability).

Repeatability = Variability / (Variability + Residual) (5)

The repeatability was calculated for all investigated US parameters using all datasets with at least 2 valid measurements (n = 47 animals). The results are summarized in table 2. Besides the TOF of the backfat layer, highest overall repeatability was obtained for the backscatter parameters. Midband fit and apparent integrated backscatter both reached values of r = .7 being considerably higher than attenuation (r = .6) and spectral slope (r = .4). Compared to the backscatter variables the analyzed cepstral parameters showed rather low repeatability (r < .4). This is in accordance to earlier investigations also stating the lowest repeatability for cepstral parameters (r < .3) (Mörlein, 2005).

Table 2: Repeatability of parameters obtained via ultrasound measurements. Given are intraclass-correlations of up to 3 measurements per animal (n = 47).

Time of Flight (backfat)	.98
Attenuation	.56
Apparent integrated backscatter	.68
Midband fit	.68
Spectral slope	.41
Excluded RF-lines	.69
Integrated cepstrum	.39
Cepstral first peak intensity	.37
Cepstral first peak position	.00

The low repeatability of the cepstral parameters may partially be explained by the low variation of those parameters between the carcasses. According to equation 5, the repeatability is calculated comparing the variations between the carcasses and between consecutive measurements on the same carcass. While the coefficient of variation between the carcasses ranged from 20 % (attenuation) to 30 % (spectral slope) for spectral parameters, the cepstral parameters showed variations in the range of 1 % (cepstrum first peak intensity) to 5 % (integrated cepstrum). Thus, the low repeatability of the cepstral parameters can be considered to be favoured by the low variation between the carcasses. Furthermore, cepstral peaks are related to the time delay between individual backscatter echoes mainly reasoned by the perimysium surrounding the muscle bundles. Consecutive measurements however will

always be performed at slightly differing positions and angles and therefore slightly differing bundle structures / orientations what may also alter the results.

As described earlier, up to 3 consecutive measurements per animal have been performed calculating mean values to at least partially overcome the low repeatability and to ensure a representative statement on carcass characteristics. To verify the influence of the use of mean values, the IMF was predicted using I) averaged acoustic values as described in the corresponding publication and II) a single measurement per animal. As a result, the accuracy of the IMF prediction via MLR using the same predictive variables (full-cross validation) decreased from R^2 = .76 to .63 with single measurement instead of mean values. Furthermore, the root mean-square errors of prediction increased from 0.34 % to 0.41 % IMF. This is comparable to earlier investigations performed on B-mode images of beef predicting the IMF. A decrease of about 50 % for the prediction error has been stated analyzing at least 4 pictures (Hassen, Wilson, Amin & Rouse, 1999).

5.2.5 Influence of ROI size and positioning

One of the important decisions to be made analyzing ultrasound data is the selection of the region of interest. Both the size of the ROI and its positioning in relation to the focus zone are important parameters potentially influencing the obtained ultrasound parameters (Gärtner, 2001; Brand, 2004). An increasing distance from the focus position is known to reduce the sound pressure of the ultrasound signal and to increase its lateral extension. Therefore, the resolution of the signal decreases with increasing distance from the focus position (Sutilov, 1984; Gärtner, 2001). Whereas the positioning of the ROI is therefore predetermined by the focus position, its size enables some variations. Earlier investigations performed on phantoms stated an ROI size of at least 1 cm in signal propagation direction to be required for reliable data (Gärtner, 2001). The width of the ROI doesn't seem to influence the results if at least 1 cm is chosen. During the present investigation an ROI size of about 5.4 cm to 1.5 cm has been used for analysis resulting in an overall muscle area of about 8.1 cm^2. In accordance with Gärtner (2001) this could be considered to be representative. However, the results published by Gärtner (2001) have been obtained analyzing a homogeneous tissue-mimicking phantom and may therefore differ from measurements on inhomogeneous tissue (e.g. muscle). Investigations performed on porcine muscle showed significant differences for spectral and cepstral parameters increasing the ROI width by 40 % (Mörlein, 2005).

To evaluate the influence of the ROI size during the present investigation, the valid measurements of all 62 carcasses were analyzed with varying ROI sizes without changing any further settings. In a first step, the ROI width was reduced to about 2/3 of the original size. Contrary to Mörlein (2005), no significant differences could be found between the mean values or standard deviations of any of the investigated ultrasound parameters. In accordance with that, all linear correlations between the variables obtained with differing ROIs ranged between r = .85 (cepstral parameters) and r = .99 (spectral parameters). Comparable findings were obtained reducing the length of the ROI in signal propagation direction by about 20 %. Again no significant differences could be found for the mean values or standard deviations of any of the investigated acoustic parameters.

These findings confirm investigations performed on tissue mimicking phantoms were no influence of the ROI size was found if a minimum size of 1 x 1 cm was used (Gärtner, 2001). However, this may vary from muscle to muscle due to the inhomogenity of the IMF distribution as discussed before. Therefore, the size of the ROI should always be chosen to obtain an area of the muscle as large as possible without deviating from to focus position.

5.3 Practical application

The previous chapters mainly described the improvements of US measurements using the obtained correction algorithms and potential influences of a wide range of factors. So far, no statements have been given targeting the practicability of the US system under slaughterhouse conditions. Therefore, the following chapter is intended to discuss practical factors like the time required for measurements or the size of the US system. Furthermore, guidelines for the approval of newly developed systems and potential improvements due to different statistical methods will be discussed.

5.3.1 Accuracy requirements

One of the most important factors for a newly developed system is the accuracy of the measurement. Strict guidelines exist for systems used to determine the lean meat percentage of porcine carcasses. According to the EU-guidelines (VO (EG) 1249, 2008) a RMSEP equal or less than 2.5 is required for LMP determination proven at least on 120 animals. No comparable guidelines however, exist for the appointment of the market value (value based

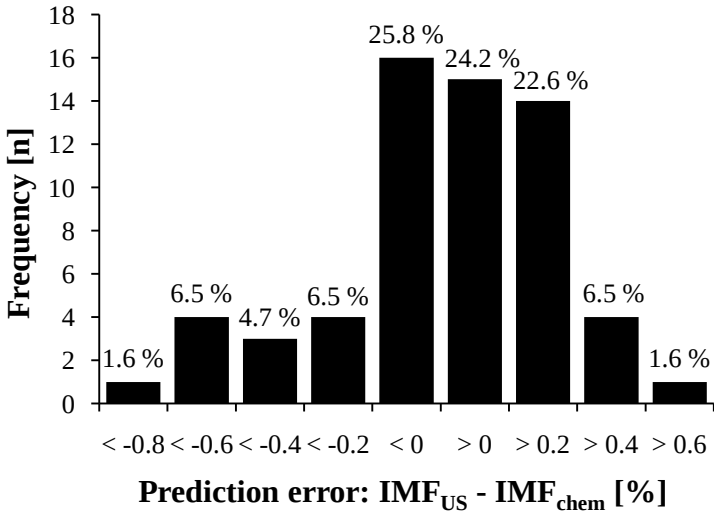


Fig. 4: Prediction errors between via ultrasound and chemical analysis determined IMF (n=62). The given values are results of a full-cross validation.

marketing). Systems like the already used AutoFOM (Carometec, Denmark) provide further information like the weight of individual parts of the carcass. In such cases, the Physikalisch-Technische Bundesanstalt (PTB) in Braunschweig can provide new systems with an accreditation.The coefficient of determination of the AutoFOM used to determine the proportion of individual sections ranges between $R^2 > 0.9$ and $R^2 > 0.74$ with RMSEP values less than 5 % (Branscheid, Honikel, Lengerken & Troeger, 2007). Besides the accuracy, some of the requirements are stability and reproducibility of the measurements. Furthermore, specific requirements can be scheduled like individual accreditations for each system due to installation differences (e.g. AutoFOM). In the present study, 50 % of the samples could be predicted with an RMSEP of 0.2 % or lower (Fig. 4), while 80 % of the samples were predicted with a RMSEP of less than 0.4 %. This is again in good accordance with earlier investigations (Mörlein, 2005). Therefore, the classification of carcasses into IMF classes was satisfying for measurements on a single position of the muscle. However, further improvements in terms of reproducibility and, correspondingly, a detailed analysis of user influences may still be requirements for an accreditation.

5.3.2 Hardware requirements

The system used for measurements during the present investigation consisted out of 3 periphery systems (US-device, function generator and personal computer), requiring some amount of personal and logistic investment. The US-device itself used a pistol grip and was therefore easy to handle with only one user. The periphery systems however, together with connections and power supplies avoided the use directly at the slaughter line. Therefore, all measurements were performed in severed areas of the slaughter facilities. This is not tenable

for practical use. Another problem comes with the time requirements. While the measurements themselves took only a matter of seconds, the data transfer required about 30 to 45 seconds per measurement. Therefore, a complete set of 3 measurements required at least 2 minutes per animal. The large setup and the time consuming data-transfer however, could be fixed with modern computer systems which should enable the production of a hardware device including all the periphery systems and also tremendously reduce the time requirements.

While an increase in processing power and some hardware modifications may be enough to fix the above mentioned problems, a major claim of ultrasound systems used for carcass classifications comes with the optimization of ultrasound parameters. The exact knowledge of size and positioning of the sound field is important for correction algorithms and the exact deduction of the related parameters. During the present investigations an Ultrafom300 (Carometec, Denmark) was used for hot carcass measurements. Due to the attachment of an acoustic lens in front of the transducer the focus position was shifted from 100 mm to about 55 mm in water. The fixed form and position of the sound field simplifies correction algorithms compared to modern medical ultrasound devices using multiple and variable sound fields. However, due to the fixation of the focus position it is adjusted to a fixed depth inside the tissue. In the present investigations, the 55 mm focus position was inside the muscle as intended for most of the measurements. However, in about 10 % of all measurements the focus position was partially placed in or near the ribs affecting the results. As the investigation targeted on a wide range of carcasses in terms of muscle and backfat width (and therefore a higher number of “extreme” animals) the effective number could be expected to be lower under “real” slaughterhouse conditions. However, problems with the ROI positioning could be avoided using flexible focus positions as already used for medical diagnostics.

To enable an accurate prediction of spectral and cepstral parameters an adequate bandwidth is required. The UltraFom300 (Carometec; Denmark) used in the present investigations had a centre frequency of 2.7 MHz and a -6 dB bandwidth ranging from 2.1 to 3.5 MHz after modification with the acoustic lens. The precision of the parameter estimation has been stated to be potentially better with higher frequencies and corresponding higher lateral resolution, while simultaneously the penetration depth decreases (Smith, 1996). Earlier investigations stated higher frequencies together with penetration depths comparable to the

present investigation, proofing the practicability of higher frequency for carcass characterization (Hein, Novakofski & O'Brien, 1992; Mörlein, Rosner, Brand, Jenderka & Wicke, 2005). Therefore, adaptations of the used frequency range may help to improve the IMF prediction. However, as for focus position and ROI the “optimal” frequency may differ dependent on the investigated animals and the required penetration depth.

Another potential adaption targets the coupling between the ultrasound device and the measured carcass. During the present investigations all measurements were performed with contact gel as coupling medium to improve the transfer of the ultrasound signal onto the carcass. Under slaughterhouse conditions, however, the use of contact gel would be both time consuming (and therefore expensive) and hygienically questionable as contact to the muscle has to be avoided. An alternative would be the use of water as coupling medium. The application on the carcass could easily be performed using a sprayer mounted directly on the US device. This would be less expensive, easy to handle and without influence on carcass quality. Further studies could help to investigate the practicability as a reduced coupling of the transducer due to the lack of coupling gel could possibly lead to a higher amount of insufficient measurements.

5.3.3 Software requirements

Besides the used hardware an important part of the present investigation included the development and improvement of the analysis software using custom made functions of Matlab R2008a (Mathworks Inc., Natick, USA). While all analyses were made single-handedly during this study, real-time IMF prediction requires a fast and fully automatic approach. As stated above for data transfer, technical advances and improved computing power will reduce the required time and also enable analysis of multiple parallel measurements and the calculation of mean values being a requirement for good IMF prediction. However, a major part of the individual processing of all datasets included the detection and removal of wrong measurements (about 38 %) which requires several adaptations to be done fully automatically. One problem is the above mentioned misplacement of the ROI (e.g. inside the bone or backfat) altering the obtained spectral parameters and rendering measurements useless. While this can easily be detected considering the corresponding B-Mode image (including the ROI), the measurements rarely showed

significantly differing acoustic parameters (e.g. AIB) and could seldom be separated from correct measurements. However, they did significantly alter the prediction model. One method to detect wrong measurements (due to weak scattering) used in the present investigation was a threshold level for the amount of deleted RF-lines. Individual signals not reaching a spectral amplitude of -40 dB were automatically discarded. If the number of deleted signals exceeded 70 %, the complete measurement was considered wrong.

Overall, an implementation of a quality control function is a major requirement for fully automatic measurements. The best option would be an analysis of the data immediately after the measurement followed by a verification of the results. This would enable additional measurements if required and avoid wrong predictive results.

5.3.4 Statistical methods

Besides the use of multiple measurements or mean values, different statistical methods may also help to improve IMF prediction. As an alternative to multiple linear regressions, partial least squares regression (PLS) may be useful. One of the advantages of PLS is the ability to handle samples with a high amount of colinearities which are affecting linear models (e.g. MLR). This is especially useful as many of the ultrasound parameters used during the recent investigations were significantly correlated with each other to a low or medium extend. In addition to the selection of the best prediction method, the used validation method should also be considered carefully. During the present investigations a full-cross validation was used. Thereby, all but one sample are used to build a calibration and the last sample is used as a test sample. This procedure is repeated until every sample is left out once. While this is a useful validation model for comparably few samples a more robust validation model would be required for an accreditation of the system. Individual sets of carcasses for calibration (at least 120 according to (VO (EG) 1249, 2008)) and validation would give a more representative conclusion about the used model. Finally, principal component analysis (PCA) could be performed allowing a more detailed description of the relationships between compositional / structural traits of the muscle (e.g. fibre diameter, dry matter) and the obtained ultrasound parameters.

5.4 Conclusion and future prospects

During this thesis, acoustic reference values were collected for porcine muscle and backfat tissue using a scanning acoustic microscope. The obtained data were implemented into the correction algorithms of a hand-held ultrasound device used to predict the intramuscular fat content in porcine *M. longissimus* on hot carcasses. Multiple linear regression allowed prediction of the IMF with an R^2 of .76 and a prediction error of 0.34 % using 3 predictive variables. Improved correction algorithms proved beneficial compared to earlier investigations at unprocessed backscatter data. Moreover, an improvement using mean values out of multiple measurements and the importance of online analysis to identify acquisition artefacts has been shown.

For further investigations, it could be interesting to evaluate the influence of handling (e.g. measurement angle and pressure) on the obtained acoustic parameters. By now, no reliable information was given about this potential error on hot carcass measurements. In addition, the analysis of multiple measurements at different positions longitudinal and cross sectional the muscle could help to overcome the high IMF variation and to improve the results. Sound fields with multiple or variable focus positions could be used to perform multiple measurements in a fast way. Finally, improved multivariate analysis (e.g. partial least squares regression) may help to increase the amount of information obtained from the acoustic parameters.

Furthermore, adjustments of software and hardware should be considered to improve the practicability. Modern computer systems could help to reduce the measurement time while quality control functions performed in real-time could reduce the amount of insufficient measurements.

6 Summary

This study aimed at improving the prediction of intramuscular fat (IMF) in porcine *musculus longissimus* via spectral analysis of the backscattered ultrasound signal on intact hot carcasses. The intramuscular fat content is considered to be one of the most important parameters influencing meat quality and its prediction was therefore the aim of multiple investigations. First attempts performed at porcine muscle via spectral analysis of unprocessed backscatter data showed promising results (prediction error (RMSEP): 0.36 %) but were not satisfying for industrial use at slaughter (Mörlein et al., 2005). One of the drawbacks of ultrasound systems is the influence of biological tissue on form and structure of the sound field which has to be corrected for quantitative analyses. Therefore, in a first step precise reference values have been collected for backfat and muscle on 27 pig carcasses representative for German slaughter facilities. Muscle and backfat samples were obtained at the position of the 2^{nd} / 3^{rd} last rib. Structural (e.g. fibre diameter) and compositional (e.g. fat, dry matter) traits were analysed in these samples under laboratory conditions. Acoustic measurements were performed using a scanning acoustic microscope with a 10-MHz centre frequency. Native muscle samples were analysed 24 hrs p.m.. In addition, formalin-fixed, frozen-thawed and aged (48 hrs p.m.) muscle samples were analysed to enable a comparison with literature data and to evaluate the influence of processing on the ultrasound parameters. The complete backfat (including skin), all 3 fat layers separately and the skin alone have been analysed. Constant environmental conditions (e.g. temperature) and exact thickness determination enabled accurate sound velocity and attenuation measurements. The obtained results were related to compositional and structural traits to evaluate their potential influence on the obtained reference values and therefore ultrasound measurements. The results can be summarised as follows:

- The obtained reference values measured for native muscle samples in phosphate-buffered saline at 38 °C were 1620 ± 5 ms^{-1} for sound velocity and 1.0 ± 0.3 dB MHz^{-1} cm^{-1} for attenuation.
- Processing of muscle samples revealed that neither storage nor freeze-thawing significantly altered sound velocity or attenuation. Fixation with formaldehyde significantly increased attenuation (2.2 ± 0.6 dB MHz^{-1} cm^{-1}).
- Attenuation has proven its usefulness to predict the IMF in muscle (r = .66) while only small relationships were found for other compositional traits (e.g. dry matter, protein content) or sound velocity.

- Ultrasound parameters of backfat were significantly different between the investigated layers. Skin showed higher sound velocity (1682 ± 23 ms^{-1}) compared to the fat layers (1436 ± 9 to 1470 ± 37 ms^{-1}). Attenuation ranged between 1.6 ± 0.7 dB MHz^{-1} cm^{-1} and 2.7 ± 1.5 dB MHz^{-1} cm^{-1} for all layers. The highest values were found in skin and the inner fat layer.
- While the sound velocity of the compound sample is mainly influenced by the backfat width (r = -.80) and the percentage amount of skin, sound velocity of individual fat layers was slightly related to dry matter (r = -.39) and the fatty acids content (r = -.37).
- Only minor relationships could be stated between attenuation and compositional traits of the individual fat layers.

The obtained results confirmed the usability of attenuation to predict the IMF in porcine muscle. Furthermore, backfat sound velocity values could directly be utilized for the optimization of correction algorithms of ultrasound measurements using a thickness dependent correction. The obtained results were implemented into the correction algorithms of a modified hand-held ultrasound device (UltraFOM 300; Carometec, Denmark) with a centre frequency of 2.7 MHz and a -6 dB frequency bandwidth between 2.1 and 3.5 MHz. Multiple measurements (up to 3) were performed on 82 hanging carcasses 45 min p.m. at the position of the 2^{nd} / 3^{rd} last rib. Out of the 218 obtained measurements, about 38 % had to be deleted due to bad coupling, ROI misplacement or insufficient signal-to-noise ratio. If at least one measurement remained per carcass, the measurement was included in the statistical analysis. If multiple measurements remained, averages were used for statistics. Overall, 62 carcasses were used for further analysis. The IMF content was chemically determined via ether extraction and correlated to ultrasound parameters which are known or expected to be related with structural alterations of the muscle induced by an increasing amount of IMF. The following results have been obtained:

- Linear correlations between IMF and the investigated ultrasound parameter are low to medium with maximum relationships to muscle attenuation (r = .61) and spectral amplitudes (r = .56).
- The low repeatability of some of the ultrasound parameters and the high cross-sectional variation of the IMF indicate that multiple measurements are necessary and the use of mean values is required for a reliable IMF prediction. In the current

study, a reduction of about 0.1 % IMF of the RMSEP compared to individual measurements was observed.

- Overall, a RMSEP of 0.34 % could be realised predicting the IMF via multiple linear regression using only variables directly available via ultrasound measurement while about 76 % of the IMF variation could be explained.
- Assigning the carcass into LOW (< 1%), MID, and HIGH (> 2 %) IMF classes resulted in about 73 % correctly classified samples proving the practicability of ultrasound to identify high quality meat.

From the given results the following can be concluded:

- Adequate correction algorithms improved the accuracy of IMF prediction in comparison with earlier investigations. In addition, due to the amplitude independency of the used variables, the method is more robust against measurement artefacts.
- Relationships between ultrasound parameters and IMF associated changes of the muscle structure have been identified.
- Additional compositional traits as analysed during our investigations (e.g. dry matter) do only slightly influence acoustic parameters.
- Multiple measurements per animal are required for sufficient IMF prediction. In addition, online analysis is required to identify artefacts during acquisition.
- Considering the cross-sectional and longitudinal variation of the IMF within the animal, the obtained RMSEP can be considered to be near to the optimum for measurements at small muscle regions.

7 Zusammenfassung

Die vorliegende Studie wurde mit dem Ziel durchgeführt, die früh postmortale Bestimmung des intramuskulären Fettgehaltes (IMF) im *musculus longissimus* warmer Schweineschlachtkörper mittels Spektralanalyse der rückgestreuten Ultraschallsignale zu verbessern. Der IMF gilt weithin als einer der wichtigsten Faktoren für die Qualität des Fleisches und ist deshalb bereits lange Gegenstand umfangreicher Studien. Erste Untersuchungen, den IMF im Rückenmuskel des Schweins mit Hilfe der Spektralanalyse von unbearbeiteten Rückstreudaten zu schätzen, zeigten vielversprechende Ergebnisse (mittlerer Schätzfehler (RMSEP): 0,36 %). Ein für industrielle Nutzung benötigter Standard konnte allerdings noch nicht erreicht werden (Mörlein et al., 2005). Einer der Nachteile von Ultraschallsystemen ist der Einfluss von biologischem Gewebe auf Form und Struktur des Schallfeldes. Diese Einflüsse müssen korrigiert werden, bevor eine korrekte Aussage über das Gewebe getroffen werden kann. In einem ersten Schritt wurden dementsprechend an 27, für deutsche Schlachthöfe repräsentativen, Schweineschlachtkörpern Referenzdaten sowohl für den Muskel, als auch den Rückenspeck gesammelt. Die Proben wurden auf Höhe der 2. / 3. letzten Rippe entnommen und ihre Struktur (z.B. Faserdurchmesser) und Zusammensetzung (z.B. IMF, Trockenmasse) untersucht. Ultraschallmessungen wurden mittels eines akustischen Mikroskops (SAM) mit einer Mittenfrequenz von 10-MHz durchgeführt. Die Muskelproben wurden sowohl im nativen (24 Std. p.m.), als auch im bearbeiteten Zustand analysiert. Hierzu wurden die Proben entweder mittels Formaldehyd fixiert, tiefgekühlt oder für weitere 24 Stunden gelagert. Die erhaltenen Ergebnisse sollten eine Abschätzung des Einflusses der Bearbeitung und darüber hinaus einen Vergleich mit der Literatur ermöglichen. Bei der Analyse der Fettschichten wurden sowohl der komplette Rückenspeck (inklusive Haut), als auch die einzelnen Fettschichten und die Haut untersucht. Konstante Umgebungsbedingungen (z.B. Temperatur) und eine genaue Dickenmessung ermöglichten eine exakte Bestimmung von Schallgeschwindigkeit und Dämpfung des untersuchten Gewebes. Um den Einfluss auf die Ultraschallparameter abschätzen zu können, wurden diese mit Struktur und Zusammensetzung des Gewebes verglichen. Die Ergebnisse lassen sich folgendermaßen zusammenfassen:

- Die erhaltenen Referenzdaten für native Muskelproben, gemessen in Phosphat-gepufferter Salzlösung bei 38 °C liegen bei 1620 ± 5 ms^{-1} für die Schallgeschwindigkeit und 1,0 ± 0,3 dB MHz^{-1} cm^{-1} für die Dämpfung.

- Die Bearbeitung der Muskelproben ergab, dass weder Lagerung noch Gefrieren einen signifikanten Einfluss auf Schallgeschwindigkeit oder Dämpfung ausüben. Fixieren mit Formaldehyd erhöhte die Dämpfung signifikant (2,2 ± 0,6 dB MHz^{-1} cm^{-1}).
- Die Nützlichkeit von Dämpfung zur Bestimmung des IMF im Muskel konnte bestätigt werden (r = ,66), während zwischen den weiteren Parametern (z.B. Trockenmasse, Protein) und der Schallgeschwindigkeit nur geringe Zusammenhänge auftraten.
- Die erhaltenen Ultraschalldaten unterschieden sich signifikant zwischen den einzelnen Schichten des Rückenspecks. Die Haut zeigte höhere Schallgeschwindigkeiten (1682 ± 23 ms^{-1}) im Vergleich zu den Fettschichten (1436 ± 9 bis 1470 ± 37 ms^{-1}). Die Dämpfung aller Schichten reichte von 1,6 ± 0,7 bis 2,7 ± 1,5 dB MHz^{-1} cm^{-1}. Die signifikant höchsten Werte erreichten hier die Haut und die innere Fettschicht.
- Während die Schallgeschwindigkeit des Rückenspecks in erster Linie von dessen Dicke (und dem Anteil der Haut) bestimmt wird (r = -,80), zeigte sich für die Schallgeschwindigkeit der einzelnen Fettschichten eine Abhängigkeit vom Anteil der Trockenmasse (r = -,39) und den Fettsäuren (r = -,37).
- Nur geringe Zusammenhänge traten zwischen der Dämpfung und der Zusammensetzung der einzelnen Fettschichten auf.

Die Ergebnisse bestätigten die Verwendbarkeit von Dämpfung zur Schätzung des IMF im Muskel. Weiterhin kann die dickenabhängige Schallgeschwindigkeit des Rückenspecks direkt für die Optimierung der Korrekturalgorithmen der Ultraschallmessungen Verwendung finden. Die erhaltenen Ergebnisse wurden in die Korrekturfunktionen eines modifizierten, tragbaren Ultraschallgerätes (UltraFOM 300; Carometec, Dänemark) mit einer Mittenfrequenz von 2,7 MHz und einem -6 dB Frequenzbereich von 2,1 bis 3,5 MHz übertragen. Hiermit wurden an insgesamt 82 hängenden Schlachtköpern (45 min p.m.) auf Höhe der 2. / 3. letzten Rippe Mehrfachmessungen (bis zu 3) durchgeführt. Von den insgesamt 218 Messungen mussten etwa 38 % auf Grund von niedriger Ankopplung, fehlerhafter Platzierung der ROI oder unzureichendem Signal-Rausch Abstand gelöscht werden. So lange mindestens ein Datensatz keine Fehler aufwies, wurde dieser für die statistische Analyse verwendet. Sobald für einen Schlachtkörper mehrere Datensätze vorlagen, wurden Mittelwerte für die Analyse herangezogen. Insgesamt konnten so 62 Schlachtkörper analysiert werden. Der IMF wurde chemisch mittels Äther-Extraktion bestimmt und mit Ultraschallparametern korreliert, bei

denen ein Zusammenhang zwischen strukturellen Eigenschaften des Muskels und des IMF bekannt ist oder vermutet wird. Die erhaltenen Ergebnisse lassen sich folgendermaßen zusammenfassen:

- Zwischen dem IMF und den untersuchten Ultraschallparametern zeigten sich niedrige bis mittlere lineare Korrelationen, wobei für die Dämpfung (r = ,61) und die integrierte Rückstreuung (r = ,56) die deutlichsten Zusammenhänge auftraten.
- Auf Grund der vergleichsweise niedrigen Wiederholbarkeit einiger Ultraschallparameter und der hohen IMF Variation innerhalb des Muskelquerschnitts war die Verwendung von Mittelwerten für eine verlässliche Bestimmung des IMF unerlässlich. Im Vergleich zu Einzelmessungen konnte so eine Reduktion des mittleren Schätzfehlers um etwa 0,1 % IMF realisiert werden.
- Insgesamt konnte mittels mehrfacher linearer Regression (MLR) ein mittlerer Schätzfehler von 0,34 % erreicht werden, wobei nur Variablen zur Verwendung kamen, die direkt mittels Ultraschallmessung zur Verfügung stehen. Ungefähr 76 % der IMF Variation konnte erklärt werden.
- Eine Einteilung der Schlachtkörper nach IMF in LOW (< 1%), MID, und HIGH (> 2 %) resultierte in etwa 73 % mittels MLR richtig geschätzter Proben. Dies bestätigt die Verwendbarkeit von Ultraschall zur Klassifizierung von Fleisch.

Aus den genannten Ergebnissen lässt sich folgendes ableiten:

- Angepasste Korrekturfunktionen verbesserten die Genauigkeit der IMF-Schätzung im Vergleich zu früheren Untersuchungen. Gleichzeitig erweist sich die Methode auf Grund der Amplitudenunabhängigkeit der verwendeten Variablen als robuster gegenüber Messartefakten.
- Zusammenhänge zwischen Ultraschallparametern und mit dem IMF assoziierte Änderungen der Muskelstruktur konnten identifiziert werden.
- Zusätzliche Gewebemerkmale, die im Rahmen der vorliegenden Studie untersucht wurden, zeigten nur minimale Einflüsse auf die ermittelten akustischen Parameter.
- Mehrfachmessungen sind für eine adäquate IMF Schätzung unverzichtbar. Darüber hinaus erweist sich eine online Analyse der Daten als nötig, um Messartefakte zu identifizieren.
- Unter Berücksichtigung der IMF Variation sowohl im Querschnitt als auch im Längsverlauf des Muskels liegt der erhaltene Schätzfehler nahe am möglichen Optimum für Messungen an vergleichbar kleinen Muskelausschnitten.

8 References

Abou El Karam, S., Buquet, B., Berge, P. and Culioli, J. (1997). Ultrasonic characterization of bovine muscles. In *Proceedings of the 43rd ICoMST. 27 July to 1 August 1997. Auckland, New Zealand* (pp. 310-311).

Abou El Karam, S., Suchorski, K., Buquet, B., Berge, P., Culioli, J., Delachartre, P., Basset, O. (2000). Effects of muscle texture on ultrasonic measurements. *Food Chemistry*, 69, 447-455.

Ahhmed, A. M., Nasu, T., Huy, D. Q., Tomisaka, Y., Kawahara, S. and Muguruma, M. (2009). Effect of microbial transglutaminase on the natural actomyosin cross-linking in chicken and beef. *Meat Science*, 82, 170-178.

Alomar, D., Gallo, C., Castaneda, M. and Fuchslocher, R. (2003). Chemical and discriminant analysis of bovine meat by near infrared reflectance spectroscopy (NIRS). *Meat Science*, 63, 441-450.

Amin, V., Patel, A., Roberts, R., Rouse, G. and Wilson, D. (1995). Application of A-mode Ultrasound for Carcass Quality Grading: A Progress Report, Iowa State University. *Department of Animal Science*, 27-31.

Baland, A. and Monin, G. (1987). Relation between muscular development and muscle collagen content in pigs. *Meat Science*, 21, 295-299.

Baldeweck, T., Laugier, P. and Berger, G. (1995). An in vitro study on porcine skin: Attenuation profile estimation using auto-regressive modeling. *IEEE Ultrason.Symp.*, 1141-1144.

Benedito, J., Carcel, J. A., Rossello, C. and Mulet, A. (2001). Composition assessment of raw meat mixtures using ultrasonics. *Meat Science*, 57, 365-370.

Blanchard, P. J., Warkup, C. C., Ellis, M., Willis, M. B. and Avery, P. (1999). The influence of the proportin of Duroc genes on growth, carcass and pork eating quality characteristics. *Animal Science*, 68, 495-501.

Bland, J. M. and Altman, D. G. (1999). Measuring agreement in method comparison studies. *Stat. Meth. in Med. Res., 8*, 135-160.

Blumer, T. N., Craig, H. B., Pierce, E. A., Smart, Jr. W. W. G. and Wise, M. B. (1962). Nature and variability of marbling deposits in *longissimus dorsi* muscle of beef carcasses. *J.Anim.Sci.*, 21, 935-942.

Brand, S. (2004). Ultraschallspektroskopie an fokussierenden Systemen unter Berücksichtigung vorgelagerter absorbierender Schichten. *Dissertation*: FB Elektrotechnik, Otto-von-Guericke-University, Magdeburg, Germany.

Brand, S., Mörlein, D. and Rosner, F. (2002). Estimation of intramuscular fat content (IMF) in the muscle longissimus dorsi (LD) of pigs by analysis of RF echo signals obtained from a clinical B-mode device. *IEEE Ultrason.Symp.*, 8-11.

Branscheid, W., Honikel, K. O., Lengerken, G. V. and Troeger, K. (2007). *Qualität von Fleisch und Fleischwaren, Band 1*. Deutscher Fachverlag GmbH, Frankfurt am Main.

Brethour, J. R. (1992). The repeatability and accuracy of ultrasound in measuring backfat of cattle. *J.Anim.Sci.*, 70, 1039-1044.

Brethour, J. R. (1994). Estimating marbling score in live cattle from ultrasound images using pattern recognition and neural network procedures. *J.Anim.Sci.*, 72, 1425-1432.

Brewer, M. S., Jensen, J., Sosnicki, A. A., Fields, B., Wilson, E. and McKeith, F. K. (2002). The effect of pig genetics on palatability, color and physical characteristics of fresh pork loin chops. *Meat Science*, 61, 249-256.

Broendum, J., Egebo, M., Agerskov, C. and Busk, H. (1998). On-line pork carcass grading with the Autofom ultrasound system. *J.Anim.Sci.*, 76, 1859-1868.

Cantrell, J. H. Jr., Goans, R. E. and Roswell, R. L. (1978). Acoustic impedance variations at burn-nonburn interfaces in porcine skin. *J.Acoust.Soc.Am.*, 64, 731-735.

Cedeno, F. O., Prieto, M. M., Espina, A. and García, J. R. (2001). Measurements of temperature and melting heat of some pure fatty acids and their binary and ternary mixtures by differential scanning calorimetry. *Thermochemica Acta*, 369, 39-50.

Chivers, R. C. and Parry, R. J. (1978). Ultrasonic velocity and attenuation in mammalian tissues. *J.Acoust.Soc.Am.*, 63, 940-953.

Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C. and Hong, K. C. (2008). The relation between glykogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. *Meat Science*, 80, 355-362.

Covington, R. C., Tuma, H. J., Grant, D. L. and Dayton, A. D. (1970). Various Chemical and Histological Characteristics of Beef Muscle as Related to Tenderness. *J.Anim.Sci.*, 30, 191-196.

Daft, C. M. and Briggs, G. A. (1989). The elastic microstructure of various tissues. *J.Acoust.Soc.Am.*, 85, 416-422.

Davanel, A., Riaublanc, A., Marchal, P. and Gandemer, G. (1999). Quality of pig adipose tissue: relationship between solid fat content and lipid composition. *Meat Science*, 51, 73-79.

Daza, A., Ruiz-Carrascal, J., Olivares, A., Menoyo, D. and López-Bote, C. J. (2007). Fatty Acids Profile of the Subcutaneous Backfat Layers from Iberian Pigs Raised Under Free-range Conditions. *Food Sci Tech Int*, 13, 135-140.

Del Grosso, V. A. and Mader, C. W. (1972). Speed of Sound in Pure Water. *J.Acoust.Soc.Am.*, 52, 1442-1446.

Dransfield, E., Martin, J. F., Bauchart, D., Abouelkaram, S., Lepetit, J. and Culioli, J. (2003). Meat quality and composition of three muscles of french cull cows and young bulls. *Animal Science*, 76, 387-399.

Essen-Gustavsson, B., Karlsson, A., Lundström, K. and Enfält, A. C. (1994). Intramuscular fat and muscle fibre lipid contents in Halothane-gene free pigs fed high or low protein diets and its relation to meat quality. *Meat Science*, 38, 269-277.

Fang, S. H., Nishimura, T. and Takahashi, K. (1999). Relationship between development of intramuscular connective tissue and toughness of pork during growth of pigs. *J.Anim.Sci.*, 77, 120-130.

Faucitano, L., Huff, P., Teuscher, F., Gariepy, C. and Wegner, J. (2005). Application of computer image analysis to measure pork marbling characteristics. *Meat Science*, 69, 537-543.

Fernandez, X., Monin, G., Talmant, A., Mourot, J. and Lebret, B. (1999). Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of *M. longissimus lumborum*. *Meat Science*, 53, 67-72.

Fiedler, I., Ender, K., Wicke, M., Maak, S., Lengerken, G. V. and Meyer, W. (1999). Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. *Meat Science*, 53, 9-15.

Fortin, A. (1986). Development of Backfat and Individual Fat Layers in the Pig and its Relationship with Carcass lean. *Meat Science*, 18, 255-270.

Fortin, A., Tong, A. K. W. and Robertson, W. M. (2004). Evaluation of three ultrasound instruments, CVT-2, UltraFom 300 and AutoFom for predicting salable meat yield and weight of lean in the primals of pork carcasses. *Meat Science*, 68, 537-549.

Gärtner, T. (2001). Ultraschallspektroskopie mit fokussierenden Systemen zur quantitativen Bestimmung von akustischen Parametern. *Dissertation*: Martin-Luther-Universität; Halle-Wittenberg.

Gelse, K., Olk, A., Eichhorn, S., Swoboda, B., Schoene, M. and Raum, K. (2010). Quantitative ultrasound biomicroscopy for the analysis of healthy and repair cartilage tissues. *Europ.Cells and Materials*, 19, 58-71.

Glueck, R. M., Mottley, J. G., Sobel, B. E., Miller, J. G. and Pérez, J. E. (1985). Changes in ultrasonic attenuation and backscatter of muscle with state of contraction. *Ultrasound in Med.& Biol.*, 11, 605-610.

Gondret, F., Combes, S., Lefaucheur, L. and Lebret, B. (2005). Effects of exercise during growth and alternative rearing systems on muscle fibers and collagen properties. *Reproduction Nutrition Development*, 45, 69-86.

Greenleaf, J. F. (1986). Tissue characterization with ultrasound (Volume I). Florida: CRC Press, ISBN: 0-8493-6221-0

Greiner, S. P., Rouse, G. H., Wilson, D. E., Cundiff, L. V. and Wheeler, T. L. (2003). The relationship between ultrasound measurements and carcass fat thickness and longissimus muscle area in beef cattle. *J.Anim.Sci.*, 81, 676-682.

Hall, C. S., Dent, C. L., Scott, M. J. and Wickline, S. A. (2000). High-frequency ultrasound detection of the temporal evolution of protein cross linking in myocardial tissue. *Ieee Transactions on Ultrasonics Ferroelectrics and Frequency Control*, 47, 1051-1058.

Hassen, A., Wilson, D., Amin, V. and Rouse, G. (1999). Repeatability of ultrasound-predicted percentage of intramuscular fat in feedlot cattle. *J.Anim.Sci.*, 77, 1335-1340.

Hassen, A., Wilson, D. E., Amin, V. R., Rouse, G. H. and Hays, C. L. (2001). Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. *J.Anim.Sci.*, 79, 11-18.

Hassen, A., Wilson, D. E. and Rouse, G. H. (2003). Estimation of genetic parameters for ultrasound-predicted percentage of intramuscular fat in Angus cattle using random regression models. *J.Anim.Sci.*, 81, 35-45.

Hein, I. A., Novakofski, J. A. and O'Brien, W. D. (1992). Ultrasound data acquisition system design for collecting high quality data from beef carcasses in the slaughterhouse environment. *IEEE Ultrason.Symp.*, 1039-1044.

Henckel, P., Oksbjerg, N., Erlandsen, E., Barton-Gade, P. and Bejerholm, C. (1997). Histo- and biochemical characteristics of the longissimus dorsi muscle in pigs and their relationships to performance and meat quality. *Meat Science*, 47, 311-321.

Herring, W. O., Kriese, L. A., Bertrand, J. K. and Crouch, J. (1998). Comparison of four real-time ultrasound systems that predict intramuscular fat in beef cattle. *J.Anim.Sci.*, 76, 364-370.

Heylen, K. (1999). Variation von anatomisch-physiologischen Merkmalen sowie Merkmalen der Fleischqualität im *M. longissimus thoracis et lumborum* des Schweins unter besonderer Berücksichtigung des intramuskulären Fettgehaltes. *Dissertation*, Institut für Tierzucht und Tierhaltung mit Tierklinik, Martin-Luther-Universität, Halle-Wittenberg

Hildrum, K. I., Wold, J. P., Vegard, H. S., Renou, J.-P. and Dufour, E. (2006). New spectroscopic techniques for on-line monitoring of meat quality. In Leo M.L.Nollet and Fidel Toldrá (Eds.) *Advanced Technologies For Meat Processing* (pp. 87-123): CRC Press.

Hintz, C. S., Coyle, E. F., Kaiser, K. K., Chi, M. M. Y. and Lowry, O. H. (1984). Comparison of Muscle Fiber Typing by Quantitative Enzyme Assays and by Myosin ATPase Staining. *The Journal of Histochemistry and Cytochemistry*, 32, 655-660.

Horák, V. (1983). A Successive Histochemical Staining for Succinate Dehydrogenase and "Reversed"-ATPase in a single Section for the Skeletal Muscle Fibre Typing. *Histochemistry*, 78, 545-553.

Insana, M. F., Zagzebski, J. A. and Madsen, E. L. (1983). Improvements in the spectral difference method for measuring ultrasonic attenuation. *Ultrasonic Imaging*, 5, 331-345.

Jenderka, K. V., Gärtner, T., Zacharias, M., Heynemann, H. & Cobet, U. (1999). System independent tissue typing of human testis and prostate. *IEEE Ultrason.Symp.*, 1141-1144; Lake Tahoe, Nevada USA.

Jorgensen, C. S., Assentoft, J. E., Knauss, D., Gregersen, H. & Briggs, G. A. D. (2001). Small intestine wall distribution of elastic stiffness measured with 500 MHz scanning acoustic microscopy. *Annals of Biomed. Eng.*, 29, 1059-1063.

Josell, A., von Seth, G. and Tornberg, E. (2003). Sensory quality and the incidence of PSE of pork in relation to crossbreed and RN phenotype. *Meat Science*, 65, 651-660.

Karlsson, A. H., Klont, R. E. and Fernandez, X. (1999). Skeletal muscle fibres as factors for pork quality. *Livestock production science*, 60, 255-269.

Kim, J. M., Lee, Y. J., Choi, Y. M., Kim, B. C., Yoo, B. H. and Hong, K. C. (2008). Possible muscle fiber characteristics in the selection for improvement in porcine lean meat production and quality. *Asian-Australian J.of Anim.Sci.*, 21, 1529-1534.

Kim, N., Amin, V., Wilson, D., Rouse, G. and Udpa, S. (1998). Ultrasound image texture analysis for characterizing intramuscular fat content of live beef cattle. *Ultrason Imaging*, 20, 191-205.

Kipfmüller, H., Bodis, K., Peschke, W. and Eichinger, H. M. (2000). Qualität von Schweinefleisch. Beeinflussung durch das intramuskuläre Fett. *Ernährungsumschau*, 47, 416-422.

Kolstad, K. (2001). Fat deposition and distribution measured by computer tomography in three genetik groups of pigs. *Livestock production science*, 67, 281-292.

Lakshmanan, S., Koch, T., Mörlein, D., Brand, S. and Raum, K. (2009). Intramuscular fat content estimation in the loin muscle of pig carcasses by ultrasound spectral parameter analysis. *IFMBE Proceedings*, Munich, Germany, 25 (13), 92-95.

Laube, S., Henning, M., Brandt, H., Kallweit, E. and Glodek, P. (2000). Die Fleischbeschaffenheit von Schweinekreuzungen mit besonderen Qualitätseigenschaften im Vergleich zum heutigen Standard- und Markenschweineangebot. *Arch.Tierz., Dummersdorf*, 43, 463-476.

Lefaucheur, L. (2010). A second look into fibre typing - Relation to meat quality. *Meat Science*, 84, 257-270.

Lengerken, G. V., Bergmann, M. and Pfeiffer, H. (1989). Bedäutung, Variabilität und Einflussfaktoren auf den intramuskulären Fettgehalt von Schweinefleisch. *Tierzucht*, 43, 385-387.

Lengerken, G. V., Schröder, C., Haugwitz, T., Berger, K. and Siegel, O. (1984). Einflüsse einer Ergänzung der Futterration durch tierische Fette auf die Schlachtkörperqualität sowie die Fett- und Fleischbeschaffenheit beim Schwein. *Archives of Animal Breeding*, 27,371-379.

Lepetit, J. (2008). Collagen contribution to meat toughness: Theoretical aspects. *Meat Science*, 80, 960-967.

Leseigneur-Meynier, A. and Gandemer, G. (1991). Lipid composition of pork muscle in relation to the metabolic type of the fibres. *Meat Science*, 29, 229-241.

Lewis, G. J. and Purslow, P. P. (1990). Connective tissue differences in the strength of cooked meat across the muscle fibre direction due to test specimen size. *Meat Science*, 28, 183-194.

Link, G. (2007). Beziehungen zwischen objektiv und subjektiv ermittelten Fleischqualitätsparametern unter besonderer Berücksichtigung der Muskelstrukturmerkmale im *Musculus longissimus* von Schweinen. *Dissertation*, Department of animal science, Georg-August-University Göttingen.

Liu, A., Nishimura, T. and Takahashi, K. (1996). Relationship between structural properties of intramuscular connective tissue and toughness of various chicken skeletal muscles. *Meat Science*, 43, 43-49.

Lizzi, F. L., Alam, S. K., Mikaelian, S., Lee, P. and Feleppa, E. J. (2006). On the statistics of ultrasonic spectral parameters. *Ultras.in Med & Biol*, 32, 1671-1685.

Lizzi, F. L., Astor, M., Liu, T., Deng, C., Coleman, D. J. and Silverman, R. H. (1997). Ultrasonic spectrum analysis for tissue assays and therapy evaluation. *Int.J.Imaging Syst.Technol.*, 8, 3-10.

Lizzi, F. L., Feleppa, E. J., Alam, S. K. and Deng, C. X. (2003). Ultrasonic spectrum analysis for tissue evaluation. *Pattern Recognition Letters*, 24, 637-658.

Lo Fiego, D. P., Santoro, P., Macchioni, P. and De Leonibus, E. (2005). Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Science*, 69, 107-114.

Madsen, E. L., Dong, F., Frank, G.R., Garra, B.S., Wear, K.A., Wilson, T., Zagzebski, J.A., Miller, H.L., Shung, K.K., Wang, S.H., Feleppa, E.J., Liu, T., O'Brien, W.D., Topp, K.A., Sanghvi, N.T., Zaitsev, A.V., Hall, T.J., Fowlkes, J.B., Kripfgans, O.D., Miller, J.G. (1999). Interlaboratory comparison of ultrasonic backscatter, attenuation, and speed measurements. *Journal of Ultrasound in Medicine*, 18, 615-631.

Maignel, L., Daigle, J. P., Gariépy, C., Wilson, D. and Sullivan, B. (2010). Prediction of intramuscular fat in live pigs using ultrasound technology and potential use in selection. *Proceedings of 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany (Nr. 0668).*

Mayne, R. and Sanderson, R. D. (1985). The extracellular-matrix of skeletal-muscle. *Collagen Related Research*, 5, 449-468.

McClements, D. J. and Povey, M. J. W. (1988). Investigation of phase transitions in glycerine/paraffin oil mixtures using ultrasound velocity measurements. *J.Amer Oil Chem Soc*, 65, 1791-1795.

McClements, D. J. and Povey, M. J. W. (1992). Ultrasonic analysis of edible fats and oils. *Ultrasonics*, 30, 383-388.

Meyer, W., Neurand, K. and Radke, B. (1982). Collagen fibre arrangement in the skin of the pig. *J.Anat.*, 134, 139-148.

Miles, C. A. and Fursey, G. A. (1977). Measurement of the fat content of meat using ultrasonic waves. *Fd.Chem.*, 2, 107-118.

Moeller, S. J. and Christian, L. L. (1998). Evaluation of the accuracy of real-time ultrasonic measurements of backfat and loin muscle area in swine using multiple statistical analysis procedures. *J.of Anim.Sci.*, 76, 2503-2514.

Monziols, M., Bonneau, M., Davanel, A. and Kouba, M. (2007). Comparison of the lipid content and fatty acid composition of intramuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science*, 76, 54-60.

Moody, W. G. and Zobrisky, S. E. (1966). Study of backfat layers of swine. *J.Anim.Sci.*, 25, 809-813.

Mörlein, D. (2005). Bestimmung des intramuskulären Fettgehaltes (IMF) im *M. longissimus* von Schweinen mittels Ultraschall. *Dissertation*, Institut für Tierzucht und Tierhaltung mit Tierklinik, Martin-Luther-Universität, Halle-Wittenberg

Mörlein, D., Rosner, F., Brand, S., Jenderka, K. V. and Wicke, M. (2005). Non-destructive estimation of the intramsucular fat content of the *longissimus* muscle of pigs by means of spectral analysis of ultrasound echo signals. *Meat Science*, 69, 187-199.

Mowafy, M. and Cassens, R. G. (1975a). Comparative study on different scalding methods and their effect on the quality of pig skin. *J.Anim.Sci.*, 41, 1291-1297.

Mowafy, M. and Cassens, R. G. (1975b). Microscopic structure of pig skin. *J.Anim.Sci.*, 41, 1281-1290.

Müller-Haye, B. (1965). Die Anwendung von Ultraschallmessungen zur Abschätzung der grobgeweblichen Zusammensetzung von Schlachthälften beim Schwein. *Dissertation*: Institut für Tierzucht und Haustiergenetik, Georg-August-Universität Göttingen.

Nair, A., Kuban, B. D., Obuchowski, N. and Vince, D. G. (2001). Assessing spectral algorithms to predict atherosclerotic plaque composition with normalized and raw intravascular ultrasound data. *Ultras.in Med & Biol*, 27, 1319-1331.

Newcom, D. W., Baas, T. J. and Lampe, J. F. (2002). Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *J.Anim.Sci.*, 80, 3046-3052.

Nguyen, C. T., Hall, C. S., Scott, M. J., Zhu, Q. Y., Marsh, J. and Wickline, S. A. (2001). Age-related alterations of cardiac tissue microstructure and material properties in Fischer 344 rats. *Ultras.in Med & Biol*, 27, 611-619.

Ninoles, L., Clemente, G., Ventanas, S. and Benedito, J. (2007). Quality assessment of Iberian pigs through backfat ultrasound characterization and fatty acid composition. *Meat Science*, 76, 102-111.

Nishimura, T., Hattori, A. and Takahashi, K. (1999). Structural Changes in Intramuscular Connective Tissue During the Fattening of Japanese Black Cattle: Effect of Marbling on Beef Tenderization. *J.Anim.Sci.*, 77, 93-104.

Nishiumi, T., Kunishima, R., Nishimura, T. and Yoshida, S. (1995). Intramuscular connective tissue components contributing to raw meat toughness in various porcine muscles. *Anim.Sci.and Tech.*, 66, 341-348.

Oelze, M. L., Zachary, J. F. and O'Brien, W. D. (2002). Characterization of tissue microstructure using ultrasonic backscatter: Theory and technique for optimization using a Gaussian form factor. *J.Acoust.Soc.Am.*, 112, 1202-1211.

Park, B., Whittaker, A. D., Miller, R. K. and Bray, D. E. (1994a). Measuring Intramsucular Fat in Beef with Ultrasonic Frequency Analysis. *J.Anim.Sci.*, 72, 117-125.

Park, B., Whittaker, A. D., Miller, R. K. and Hale, D. S. (1994b). Predicting Intramuscular Fat in Beef Longissimus Muscle from Speed of Sound. *J.Anim.Sci.*, 72, 109-116.

Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillespie, C. A. and Stempel, K. E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Histochemical Journal*, 13, 319-327.

Purslow, P. P. (2002). The structure and functional significance of variations in the connective tissue within muscle. *Comp.Biochem.& Phys.*, 133, 947-966.

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, 70, 435-447.

Raum, K. (2008). Microelastic imaging of bone. *IEEE Trans Ultrason Ferroelectr Freq Control.*, 55, 1417-1431.

Rehfeldt, C., Tuchscherer, A., Hartung, M. and Kuhn, G. (2007). A second look at the influence of birth weight on carcass and meat quality in pigs. *Meat Science*, 78, 170-175.

Reichard, W., Müller, S., Schuster, M. and Peschke, W. (1998). Results of a comparative study about methods for analysis of intramuscular fat content in pig longissimus dorsi muscle by extraction. *Archives of Animal Breeding*, 41, 167-178.

Ripoll, G., Joy, M., Alvarez-Rodriguez, J., Sanz, A. and Teixeira, A. (2010). Estimation of light lamb carcass composition by in vivo real-time ultrasonography at four anatomical locations. *J.Anim.Sci.*, 87, 1455-1463.

Roberjot, V., Laugier, P. and Berger, G. (1994). Anisotropy in bovine skeletal muscle in vitro: Frequency dependent attenuation and backscatter coefficient over a wide range of frequencies. *Proceedings of Ultrasonics Symposium*, 1467-1470.

Ryu, Y. C. and Kim, B. C. (2005). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig *longissimus dorsi* muscle. *Meat Science*, 71, 351-357.

Saijo, Y., Sasaki, H., Okawai, H., Nitta, S. and Tanaka, M. (1998). Acoustic properties of atherosclerosis of human aorta obtained with high-frequency ultrasound. *Ultrasound in Med.& Biol.*, 24, 1061-1064.

Sapin-de Brosses, E., Gennisson, J.-L., Pernot, M., Fink, M. and Tanter, M. (2010). Temperature dependence of the shear modulus of soft tissues assessed by ultrasound. *Phys.Med.Biol.*, 55, 1701-1718.

Scheipers, U., Ermert, H., Sommerfeld, H. J., Garcia-Schürmann, M., Senge, T. and Philippou, S. (2003). Ultrasonic multifeature tissue characterization for prostata diagnostics. *Ultras.in Med & Biol*, 29, 1137-1149.

Smith, N. B. (1996). Effect of myofibril length and tissue constituents on acoustic propagation properties of muscle. *Ph.D.Thesis, University of Illinois.*

Sorland,G. H., Larsen, P. M., Lundby, F., Rudi, A. P. and Guiheneuf, T. (2004). Determination of total fat and moisture content in meat using low field NMR. *Meat Science*, 66, 543-550.

Sutilov, V. A. (1984). Physik des Ultraschalls. Berlin: Akademie-Verlag. ISBN: 3-2118-1798-0

Suzuki, K., Irie, M., Kadowaki, H., Shibata, T., Kumagai, M. and Nishida, A. (2005). Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. *J.Anim.Sci.*, 83, 2058-2065.

Topp, K. A. and O'Brien, W. D. Jr. (2000). Anisotropy of ultrasonic propagation and scattering properties in fresh rat skeletal muscle in vitro. *J.Acoust.Soc.Am.*, 107, 1027-1033.

Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. *Meat Science*, 70, 493-508.

Towa, R. T., Miller, R. J., Frizzell, L. A., Zachary, J. F. and O'Brien, W. D. (2002). Attenuation coefficient and Propagation Speed Estimates of Rat and Pig Intercostal Tissue as a Function of Temperature. *IEEE Transactions on UFFC*, 49, 1411-1420.

Unruh, J. A., Friesen,K.G., Stuewe,S.R., Dunn,B.L., Nelssen,J.L., Goodband,R.D., Tokach,M.D. (1996). The influence of genotype, sex, and dietary lysine on pork subprimal cut yields and carcass quality of pigs fed to either 104 or 127 kilograms. *J.Anim.Sci.*, 74, 1274-1283.

van Laack, R. L., Stevens, S. G. and Stalder, K. J. (2001). The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. *J.Anim.Sci.*, 79, 392-397.

Ville, H., Rombouts, G., Van Hecke, P., Perremans, S., Maes, G., Spincemaille, G., Geers, R. (1997). An evaluation of ultrasound and nuclear magnetic resonance spectroscopy to measure in vivo intramuscular fat content of longissimus muscle of pigs. *J.Anim.Sci.*, 75, 2942-2949.

Villegas, F. J., Hedrick, H. B., Veum, T. L., McFate, K. L. and Bailey, M. E. (1973). Effect of diet and breed on fatty acid composition of porcine adipose tissue. *J.Anim.Sci.*, 36, 663-668.

VO (EG) 1249 (2008). Verordnung (EG) Nr. 1249/2008 der Kommision vom 10. Dezember 2008 mit Durchführungsbestimmungen zu den gemeinschaftlichen Handelsklassenschemata für Schlachtkörper von Rindern, Schweinen und Schafen und zur Feststellung der diesbezüglichen Preise.

Wagner, J. R., Schinckel, A. P., Chen, W., Forrest, J. C. and Coe, B. L. (1999). Analysis of body composition changes of swine during growth and development. *J.Anim.Sci.*, 77, 1442-66.

Warentest (2008). Warentest für Mastferkel 2008 Prüfungsbericht. *Münster, Landwirtschaftliches Wochenblatt Westfalen-Lippe.*

Whittaker, A. D., Park, B., Thane, B. R., Miller, R. K. and Savell, J. W. (1992). Principles of Ultrasound and Measurement of Intramuscular Fat. *J.Anim.Sci.*, 70, 942-952.

Wicke, M. (1989). Einfluss einer divergenten Selektion nach Merkmalen der Muskelstruktur des M. longissimus dorsi auf die Belastungsempfindlichkeit und die Schlachtkörperqualität des Schweines. *Dissertation*, Karl-Marx Universität, Leipzig

Wood, J. D. (1973). The fatty acid composition of backfat from Pietrain and Large White pigs. *Anim.Prod.*, 17, 281-285.

Wood, J. D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., Whittington, F.M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343-358.

Wood, J. D., Enser, M., Whittington, F. M., Moncrieff, C. B. and Kempster, A. J. (1989). Backfat Composition in Pigs: Differences Between Fat Thickness Groups and Sexes. *Livestock production science*, 22, 351-362.

Wood, J. D., Nute, G.R., Richardson, R.I., Whittington, F.M., Southwood, O., Plastow, G., Mansbridge, R., da Costa, N., Chang, K.C. (2004). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, 67, 651-667.

Zaidman, C. M., Holland, M. R., Anderson, C. C. and Pestronk, A. (2008). Calibrated quantitive ultrasound imaging of skeletal muscle using backscatter analysis. *Muscle & Nerve*, 38, 393-398.

Zgur, S. (1991). Histologische und biochemische Veränderungen im Muskelgewebe wachsender Schweine. *Dissertation*: Landwirtschaft. Fakultät, Georg-August-Universität, Göttingen

Erklärungen

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

Göttingen, den ……..

……..
(Tim Koch)

2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Göttingen, den ……..

……..
(Tim Koch)

Lebenslauf

Persönliche Daten:

Geburtsdatum:	09. März 1980
Geburtsort:	69412 Eberbach
Staatsangehörigkeit:	deutsch
Familienstand:	verheiratet

Schulbildung:	August 1999	Abitur, Hohenstaufen-Gymnasium Eberbach
Zivildienst:	07.1999 – 08.2000	Zivildienst beim Deutschen Roten Kreuz Eberbach, Ausbildung zum Rettungshelfer
Studium:	10.2000 – 09.2006	Studium der Biologie, Ruprecht-Karls Universität Heidelberg; Abschluss: Diplom
Beruflicher Werdegang:	10.2006 – 11.2006	Mitarbeiter Randstad Deutschland
	01.2007 – 12.2009	Wissenschaftlicher Mitarbeiter im Department für Nutztierwissenschaften Göttingen Projekt: „Ultrasound backscatter model based assessment of porcine meat“
	04.01.10 – 06.2010	Wissenschaftlicher Mitarbeiter Charité Berlin
	01.10. 10 – 31.12.10	Wissenschaftlicher Mitarbeiter im Department für Nutztierwissenschaften Göttingen

Danksagung

Hiermit möchte ich all jenen herzlich danken, die auf verschiedenste Weise zum Gelingen dieser Arbeit beigetragen haben.

In erster Linie gilt mein Dank natürlich Prof. Dr. Michael Wicke für seine Betreuung des Dissertationsvorhabens und Prof. Dr. Kay Raum für die Übernahme des Korreferats und die vielfältige Unterstützung bei Fragen jedweder Art. Weiterhin danke ich Prof. Dr. Dr. Claus-Peter Czerny für die Übernahme der Nebenfachprüfung.

Ein großer Dank gilt der gesamten Arbeitsgruppe „Produktkunde – Qualität tierischer Erzeugnisse“ für die angenehme Arbeitsatmosphäre und die entgegengebrachte Hilfe. Besonders danke ich hierbei Dr. Daniel Mörlein für viele Stunden konstruktiver Gespräche und seinen nie endenden Ideenreichtum. Weiterhin danke ich Dipl. Biol. Sabine Janisch für das gute Zusammenleben im Büro und Peter Ludewig für seine unermüdliche Hilfe bei jeder Art von Problemen.

Der Arbeitsgruppe „Ultraschallspektroskopie und akustische Mikroskopie; Charité, Berlin“ danke ich für die umfangreichen Arbeiten und die entgegengebrachte Unterstützung im Bereich Ultraschall.

Neben all jenen Menschen die ich im Verlauf meiner Arbeit kennen lernen durfte gibt es auch jene, die von außerhalb große Teile zum Gelingen dieser Arbeit beigetragen haben.

Hierbei sind natürlich in erster Linie meine Eltern, Horst und Ulrike sowie meine Schwester Tina zu nennen deren Liebe und Unterstützung, sowie ihr nie endender Glaube an meine Arbeit über all die Zeit diese erst ermöglicht haben.

Ich danke meiner Familie und all meinen Freunden für viele schöne Jahre.

Schließlich möchte ich auch jener Person danken die mein Leben in den letzten Jahren um so vieles bereichert hat. Ich danke meiner Frau Ramona für ungezählte schöne Stunden, für ihr offenes Ohr bei all meinen Problemen und dafür, dass sie auch in schweren Zeiten immer zu mir steht.

An all jene Menschen mein herzlichster Dank !